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THE SOIL-BLOCK WASHING METHOD IN QUANTITATIVE ROOT STUDY[1]

BY T. K. PAVLYCHENKO[2]

Abstract

It has been observed consistently that competition among plants first takes place between the root systems, and that the nature, vigor, extent and distribution of the root systems have an important bearing on the development of top growth. A new technique for root studies, the Soil-block Washing Method, is described in considerable detail. This method enables the investigator to procure entire root systems at any stage of plant development, from plants grown under natural soil conditions.

Introduction

The root systems of plants have received less attention than the above-ground parts, largely because the latter are conspicuous, have definite economic value in many cases, and form an easily available source of material for study. As a result knowledge of root systems was scarce and inaccurate for a long time, and today it is still inadequate.

Since the eighteenth century, many attempts have been made to secure the root systems of various wild and cultivated species in sufficiently sound condition to allow of study of their nature and extent. As a result of such work considerable light has been thrown on a hitherto obscure phase of the plant's development. Now it is well established that under all conditions the root systems perform two distinct functions. The first is essentially physiological in nature. This relates to supplying the plant organisms with food substances from the soil solution or with storage of the food reserves manufactured within the plants. The second is purely mechanical and effects the anchorage of plants in the growing medium (10). If either function or both are disturbed the effect is decrease of the top growth or the ultimate death of the plant. On the other hand, plants with healthy and extensive root systems develop very strong top growth (25) capable of resisting a surprisingly great amount of hardship and injury imposed by such conditions as heavy winds, direct heat, mechanical cutting, grazing, or tramping. Besides these functions, the root systems under all conditions play a leading part in keeping the plant in the best possible balance with the environment.

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Upon germination, for instance, roots are the first visible organs. They also are first to adapt themselves to various soil conditions before the stem successfully emerges above the surface (26). During the plant's life roots must continuously adjust themselves to the changing conditions in the ground, as otherwise the top growth will perish (24). Root systems of various plant species and varieties respond differently toward deep and shallow, heavy and light, dry and moist soils (12, 14, 20, 39). Similar variations are observed with respect to their behavior toward the application of different mineral fertilizers (31). The susceptibility of plants to damage from different root-rot diseases and insect pests also is governed to a considerable extent by their ability to regenerate the damaged roots or to replace them by new ones (34).

The above considerations, based on abundant experimental evidence, indicate that the root systems are always the first part of the plant to be profoundly influenced by the entire complex of growth factors. Consequently the top growth, which depends directly upon the performing capacity of the roots, is only a true resultant of the interaction between the growth factors in a given habitat and the root systems of the plants subjected to their direct and continuous influence. Therefore it is only logical to expect that adaptation of species and varieties to ecologically different regions depends largely upon the abilities of their roots to thrive under many environmental conditions. If the soil temperature or moisture conditions of a habitat are detrimental to the roots of an introduced species, it will never become established. Plants with roots that barely survive the test usually remain in the district, but only as subdominant vegetation without economic significance. On the other hand, plants with roots well fitted to the new environment thrive, and in time become ecotypes of the habitat. They soon assert their positive (successful crop introductions) or negative (successful weed invaders) influence upon the country in accordance with their economic value.

In view of these facts it was thought that a quantitative study of root systems extricated from their natural environment in the least damaged condition undoubtedly would provide information fundamental to the understanding of the plant's top development. Such information may be of value in such phases of agricultural investigation as soils, weed control, field management, plant ecology, plant breeding, plant pathology and entomology. The results also may prove to be of particular importance in practical work concerning the rehabilitation and reclamation of land by amelioration projects, such as irrigation (12) or the assigning of certain areas to grass production. The reliability of results from a root study, however, depends largely upon the technique used. Therefore an attempt has been made here to give at least a brief systematic review of the literature dealing with the subject as well as to describe and discuss adequately the technique developed in the quantitative root studies at the University of Saskatchewan. In the review of literature the methods have been grouped on the basis of similarity of essential features.

Methods Previously Used in Root Studies

1. Direct Washing Method

Hales was perhaps the first to comprehend fully the value of information on the extent of the root systems of agricultural plants from the standpoint of crop production. He made an attempt to obtain root systems of some agricultural plants as early as 1727 (6). His simple method consisted of a direct washing from the ground. Apparently it was a long and awkward procedure, because it found no followers among his contemporaries and was soon abandoned by its originator.

2. Trench-washing Method

In 1855 Schubart (29) followed a different procedure. This may be properly called the "Trench-washing Method". He first dug a trench beside the plant or plants selected for the study and then washed out the root systems directly from a vertical wall of the trench. The method enabled him to determine the depth of penetration and to procure in bulk root material of such crops as wheat, winter rape and clover. However, this method does not include means whereby the root systems of individual plants may be separated from the material and studied in detail. The method, therefore, is better adapted to investigating qualities of root material than to quantitative studies of the root systems of individual plants. Schubart's method was followed more or less closely by Goff (4), Morrow and Hunt (21) and others.

3. Water-culture and Soil-containers Method

Nobbe (22) was interested in the effects of different mineral salts upon the development of root systems and their structures. He realized the difficulty in carrying out investigations with plants grown in the natural undisturbed ground and used large glass cylinders filled with certain kinds of soil mixtures. He grew the seedlings in water cultures and then transplanted them into the cylinders. At definite stages of plant development the entire content of the cylinders was removed and the root systems liberated from earth by soaking in water and shaking with the fingers. Kraus (16, 17) used pots and wooden boxes for the same purpose. Hoveler (11) and Frank (3) studied corn, peas and beans in vessels filled with various soils. Tucker and von Seelhorst (37) investigated the root systems of oat plants grown in zinc cans containing about 40 lb. of soil. Arker (1) worked with *Lupinus* and sunflower in either water cultures or soil containers. Very extensive study of the root systems of wheat, oats and barley by von Seelhorst and Freckmann (32) was entirely carried out in pots. Mielecki (19) used water cultures exclusively in studying effects of potash on the development of the root systems of different plants.

By growing plants in the various kinds of soil containers and washing the roots with water, or by using water cultures, the workers were in a position to secure root material and sometimes even root systems of single plants in good condition without much effort. The method is cheap, quick and easy. However, the results obtained under such highly artificial conditions, although

of theoretical interest, do not illustrate the usual extent, shape, penetration, branching, and consequently performance, of the root systems, which the latter exert when grown in the natural habitats.

4. Hellriegel's Steel Cylinder Method

In 1883 Hellriegel (9) described a very interesting method for studying roots of plants grown in the open fields. He used steel cylinders about 400 sq. cm. in cross section and as long as required by the material studied. The implement was first driven into the ground to the depth desired. Then the earth around it was removed and the base of the soil column inside the cylinder was examined with respect to the number of root stubs found at that level. Von Seelhorst (31) and other workers also used this method in several experiments. The method, however, is suitable only for studying depth of penetration and sometimes the lateral spread of the root systems.

5. Steel-frame Washing Method

In studying the root systems of corn plants, Hays (7) used steel frames constructed of water pipes one inch in diameter. At intervals of 2–3 inches in depth, two-inch wire netting was fastened across the frame in order to provide supports for the roots. The frame was set in the ground, filled with sifted soil, and corn planted in the centre of it. At certain stages of growth the frames were excavated and the loose soil washed away, leaving the root system hanging over the wire netting inside the steel structure.

6. Soil-prism Washing Method

Following the principles of Hays' method, King (15) developed a procedure, differing only in that the plants were established in the undisturbed soil and grew under natural field conditions until the stage at which they were to be studied. Then a prism of soil one foot thick and as long and deep as desired was dug, and reinforced by a steel or wooden frame very similar to that constructed by Hays. The wire netting was stretched over the four vertical sides of the frame to keep the soil column intact. Many thin rods driven through the prism were substituted for the wire netting used by Hays. The loose dirt from the top of the prism was removed and replaced by a layer of plaster of Paris in order to fix the top growth in a permanent position. Soil from the prism was gradually removed by means of water, washing proceeding from the top down. The root systems thus freed of earth were suspended from the layer of plaster of Paris and held in their relative positions by the cross rods. King's method has been used by Ten Eyck (36), Goff (5) and Shepperd (33). Besides being extremely muddy and slow (as one has to work in the hole flooded with water and thin mud), the method had several other important disadvantages. First, owing to the fact that the washing started from the top of the prism, the younger, weak roots and particularly fine root branches, which are the most important water absorption structures, were usually completely lost, being torn away by clods of soil collapsing under the pressure of the water spray. Second, the prisms usually were too narrow, and therefore the root material obtained from them represented only a cross section through the root systems, rather than the root systems in their entirety.

Otuka (23), in his root study of the apple tree, considerably modified the prism washing method by discarding the steel frame and cross rods used by King. Part of an orchard was fully excavated, a number of soil prisms of definite size and shape being left at intervals over the excavated area. These were then encased in wooden boxes to prevent roots from extending beyond the prism walls. After this the entire excavation was filled in and the surface leveled. Approximately 10 apple seeds were planted in each prism. At certain stages of plant development the soil and wooden boxes around each prism were removed and the earth washed away as in King's method.

7. Concrete-compartment Washing Method

Schulze (30) was interested in extricating entire root systems from the ground. Realizing the difficulty of the task with plants grown in the undisturbed ground, he constructed a number of separate compartments each $24 \times 24 \times 80$ inches in size. Three walls of each compartment were made of concrete, while the fourth side opened into a room common to all compartments. The open side was then closed by two metal plates. The inner plate was perforated with holes about $\frac{3}{8}$ in. in diameter, while the outside one was solid in order to make the compartments water-tight. The compartments were packed with sifted soil and sown to different crops. At various stages of plant growth the root systems were studied in the following manner: A stream of water was used to remove the loose soil, which was carried off through the perforated plate, the solid plate having been taken away before washing started. In spite of constructional differences the method closely resembles that of Hays as in both cases sifted soil was used instead of the natural ground.

8. Nail and Needle Brush Washing Methods

Introduced by Rotmistroff (27) and modified by Maschhaupt (18) and Spirhanzl (35) this method is designed to keep the roots in their original position after the earth is removed by water, and thus to disclose a true picture of their distribution under the surface. The principal features of the method as used by Rotmistroff may be described as follows: a wooden box only one inch wide but about 28 inches long and 60 inches deep was packed with sifted soil and set into the ground. In this narrow box plants grew to a certain stage of development. Then the box was excavated and one side board replaced by a zinc plate filled with one inch nails to form a sort of nail brush. Loose dirt from the box was washed off by a stream of water and roots were left held in place by the nails of the zinc brush. Maschhaupt applied a similar principle but worked with plants grown in the field. The brush in this case consisted of a large board filled with long needles. This was forced against the vertical wall of a trench dug on one side of the plants selected for the study. In addition he used a large steel sheet, which he forced into the ground at the desired distance in front of the needle brush. The washing was done as in Rotmistroff's method.

Spirhanzl constructed a nail brush different from either of those mentioned. Instead of a solid board he used a screen made of wooden slats and drove the nails into it where the slats crossed. Water was run against the wall face to be washed through the square holes between the slats. After a thorough trial this method proved to be unsatisfactory and was discarded. Spirhanzl then used his brush to wash the earth from blocks of soil fully excavated and brought to the surface. In this case the results were more satisfactory, yet it took as much as 13 days of continuous work to remove the dirt from a comparatively small block. He concluded, therefore, that a satisfactory method for root study still remained to be found.

9. Observation-pit Method

Rotmistroff (28) developed also an interesting observational method for root study. It consisted of a pit 27 feet long, 3 feet wide and 4 feet deep, dug along one side of a plot where the penetration of roots was to be observed. In a vertical wall of the pit horizontal holes two inches high, four inches wide and eight inches deep, were made. These were spaced 20 inches apart on the horizontal lines and 4 inches apart on the vertical lines. In the course of penetration, roots met the holes at different depths and their tips projected through the upper walls. These were observed and the depths recorded. To prevent the holes from drying out they were plugged with wooden blocks. The vertical wall of the pit was covered with a thick sheet of asbestos paper and, in addition, the entire pit was protected by boards and thick straw mats.

From time to time the protections were removed and observations made. To determine the lateral spread of roots, similar holes dug vertically at certain intervals from one another have been used, successive holes being dug at a greater distance from the row of plants under observation. It is claimed that the tips of the lateral roots could be easily observed and the greatest spread accurately determined.

10. Direct Tracing of the Main Roots

By the end of the nineteenth century, some workers began to realize that all the attempts so far made to procure, in their entirety, the root systems of plants growing in the undisturbed ground, usually produced incomplete and badly dislocated root material. This suggested that it would be advisable to sacrifice some fine root structures and to study the main roots in their exact positions as observed when they were individually traced in the ground with sharp pointed instruments. In this manner Headden (8), in 1896, examined the root systems of six-year-old alfalfa plants and determined the greatest depth of penetration. Six years later, Cottrell (2) traced the main roots of an eight-year-old alfalfa plant in the ground to a depth of more than 10 feet. The technique used by these two workers, however, was not sufficiently stabilized and although it certainly signalized a definite turning point in the efforts in root study, it can hardly be regarded as a distinct method.

11. Weaver's Method

The principle of tracing roots directly in the ground was also adopted by Weaver of Nebraska in his extensive ecological researches with native and cultivated plant species. He clearly described individual phases of the work, and produced a definite method in root study, which now is generally known as Weaver's trench-tracing method. Weaver (38) has briefly described the method as follows: "The methods employed in excavating root systems was to dig trenches 2 to 3 feet wide and 6 to 10 feet long to a depth of about 6 feet by the side of the plants to be examined. This offered an open face into which one might dig with a hand pick furnished with a cutting edge on one end and, after sufficient practice and acquaintance with the soil texture, successfully excavate a root system almost in its entirety." Owing to the skill and perseverence with which this method has been used by Weaver and his co-workers over a long period of years, it has been the dominant method in root study and is so at the present time.

In Canada Weaver's method has been consistently followed by Simmonds and his co-workers in the study of root-rot disease of wheat (34).

Soil-block Washing Method

1. General Considerations

The objective in root study of any kind should be to determine exactly the underground development with respect to at least one of the following three cardinal points:

(a) The habit of root growth as indicated by the natural spread and course of penetration of roots.

(b) The quantity of root material found at different ground levels.

(c) The performing capacity of root systems as indicated chiefly by the amount, extent, and location of the fine root branches on the main roots of each species.

2. Historical

It is well to keep in mind that the method herein described was developed, not from a special project outlined for the purpose, but as a necessity in work primarily concerned with the competitive efficiencies of different species grown together under ordinary field conditions. In this study it was observed that the performance of the aboveground growth of crop and weed species was always governed by the condition and extent of their root systems. It is obvious, therefore, that in a study of this kind, information concerning the three above-mentioned points is essential. To obtain such information on the root systems of the plants studied, several methods previously used by different workers were tried, but with unsatisfactory results. It became evident that an altogether different process for obtaining representative samples of the root systems was needed. Such a process was eventually found and is described here as the Soil-block Washing Method.

3. Principles Underlying the Method

The method was devised to meet the following requirements:

(1) Extrication of root systems of plants grown under ordinary field conditions in various types of soil, with the finest young branches in undamaged condition, at any stage of plant development.

(2) Removal of large quantities of earth from the blocks in a reasonably short time, thus reducing cost and providing an opportunity for comparative study of several plant species at the same time.

(3) Supplying root material in such condition that the root systems of individual plants of various species grown in competition may be separated and studied in detail.

4. Plant Material

During the first year of this study, plant material grown both in various soil containers in the greenhouse and in the open field, was extensively used. The results obtained from the two sources had adequately demonstrated that the root systems of the plants, grown even under most favorable greenhouse conditions, never attained more than 60% of the growth attained by the same species under comparatively poor environment in the open field. Considering this fact together with the desirability of procuring results directly applicable to ordinary farming conditions, it was decided in 1931 to discontinue work with plants grown in artificial media.

Experience has shown that practically all cereals and the majority of forage crops as well as annual, biennial and many perennial weed species make excellent material for root study with the soil-block washing method. Only perennials with extensive side runners cannot be studied by this method at the later stages of their development. In spring the selected species are seeded on a piece of land which has been previously treated in the same manner as the ordinary farm fields. Usually a number of single plants and several plots of each crop free from weeds (checks), and in competition with weeds (competition plots), are seeded the same day. Seeding in the check and competition plots is most conveniently done with the Columbia hand drill at ordinary rates of seeding and with rows spaced six inches apart. In the competition plots, weeds are sown between the crop rows with the same drill at the rate desired but much shallower than the cereals and at about the same depth as the forage crop seeds. The single plants are sown each in the centre of a plot ten feet square to eliminate competition of any kind.

5. Excavations

To follow the gradual progress in root development of each species from emergence to maturity it is necessary to make excavations of plants that emerged on the same date, at several stages of their development. With annual species it was found that excavations at 5, 22 and 40 days after emergence and one at maturity give a sufficiently complete picture of the entire

process of root increase during their life period. In biennial plants excavations made at 5, 22, 40 and 100 days after emergence, and in the next year one in May and one before maturity were sufficient. Perennial plants should be excavated at the same stages as biennials during the first year and, in addition, once in August of the second year, and again in August of the third year of growth.

6. Size of Soil Blocks

The soil blocks are cut in such sizes as to include the entire mass occupied by the root systems at each stage of growth. Dimensions of the blocks vary with the plant material, type of soil, amount and distribution of moisture in the ground, and many other factors. For this reason the sizes of blocks cannot be stated dogmatically for all conditions. Under the conditions prevailing at Saskatoon, blocks 14 × 14 × 14 inches have been large enough to include entire root systems, at the five-days stage, of all the cereal crops studied over a period of six years. Forage crops and weeds invariably take smaller blocks at this particular stage. For 22-day material blocks 24 × 24 × 32 inches have proved to be sufficiently large. At the 40-day stage blocks 26 × 26 × 46 inches have frequently been required. At maturity, cereal crops take blocks

FIG. 1. *A block of soil 40 × 40 × 70 inches completely cut out and ready for encasing.*

up to 32 × 32 × 66 inches. Forage crop and weed plants at the later stages of growth present special difficulty with respect to the volume of the blocks needed. It is sufficient to say that two- and three-year-old plants of some grasses have been removed in blocks as large as 40 × 40 × 70 inches and yet some roots were partially severed by the spade before encasing.

The excavations are made according to the time table based on the date of emergence of each species, and cover the whole series of blocks to be studied. The size of a block is first marked off on the field surface. Around this rectangle a trench is dug to the depth required. At one side, the trench is made at least 12 inches wider than the depth of a block in order to provide sufficient space for the block to be conveniently tipped over before lifting. A block fully prepared for encasing is illustrated in Fig. 1.

7. Encasing

Proper encasing is of primary importance in this method. Without this precaution there is always danger of damaging the block either in elevating it from the hole or in transit to the place where the soil is to be removed from the roots. Wooden encasing frames are inexpensive and fully satisfactory.

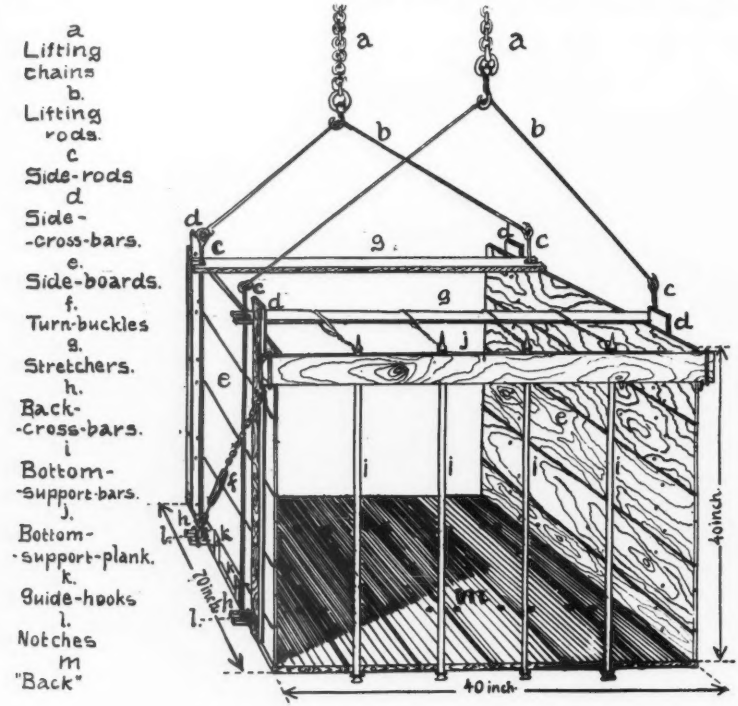

FIG. 2. *Diagram of the encasing frame.*

Fig. 2 presents a diagrammatic view of the encasing frame and Fig. 3 illustrates it in use. For small blocks, all sides of the encasing frame are made of one-inch lumber. For blocks weighing over 500 lb. the backs (*m*, Fig. 2) of the frames are constructed of two-inch material. The side-bars *(d)* are made of 2 × 4 in. material regardless of the size of the block. In all cases where the weight of a block exceeds one ton, the cross bars of the backs *(h)* should be made of heavy steel. The back *(m)* of the frame is fixed first against that side of the block which faces the wide part of the trench (front in Fig. 3). Then the side boards are fastened to the back with four $2\frac{1}{2}$ in. nails driven through the holes of the guide hooks on the back *(k)*. The next step is to tighten up the frame over the block. This is done by twisting the two- or three-ply hay wire catching projecting ends of the cross bars of the side boards. After this the bottom of the block is reinforced by driving three or four steel bars *(i)* of a suitable strength on the level with the bottom end of the back. The bars are long enough to go completely through the block and project at least two inches beyond its front side. The ends of the bars

FIG. 3. *A block of the same size as shown in Fig. 1, fully encased.*

in front and at the back of the block are caught by wires and tightly fastened to the front lower stretcher *(g)* and the back lower cross bar *(h)*. With very large blocks weighing from one to four tons, a piece of 2 × 8 in. plank of proper length is put under the front ends of the bottom bars, caught by chains equipped with hooks and turnbuckles, and tightly fastened to the upper cross bar of the back *(h)*. The block thus reinforced is then carefully tipped over on its back in the hole.

8. Elevating the Blocks

In this position the block is ready to be raised to the surface. Four steel side-rods *(c)* are hooked on the special notches *(l)* in the cross bars of the back. The top ends of the side-rods then are connected in pairs by the lifting rods *(b)* to provide a suitable grip for the lifting hook of a block and tackle. To avoid possible damage to the block from the squeezing action of the side rods, wooden stretchers *(g)* are fitted in between each pair of the side rods to keep them apart at the distance of the width of the block. One or two strong wooden tripods, not less than 12 feet in height, and blocks and tackles of the required strength are used to raise the block to the surface.

FIG. 4. *The adjustable nozzle for use in washing out the roots.*

9. Washing

In the past the main reason why the root systems were always so seriously damaged in the extricating process was that the force of the water used to dislodge the soil particles from the roots was too great for the tender root structures to withstand without breaking. This was also particularly true of methods used to remove the mass of the soil in a dry condition. It has been found, however, that the most delicate root structures can endure a surprisingly large amount of water action if the latter is applied properly. For this reason, in the soil-block washing method, the process of liberating the root material from the earth is done exclusively by water from a nozzle specially constructed for this purpose. This is shown in Fig. 4.

No root is touched by the hands or any other hard object until the last particle of soil is removed from the root system. To make the soil more susceptible to the action of water prior to washing, the block is thoroughly soaked in a large tank filled with water. Fig. 5 presents a general view of

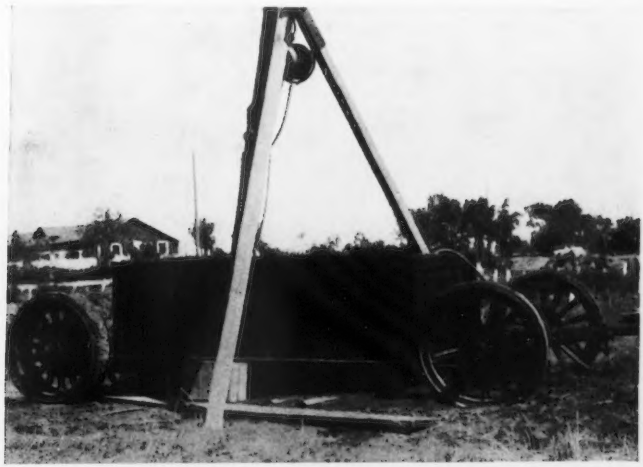

FIG. 5. *A soaking tank 2½ × 4 × 8 feet.*

the equipment. The tank is water-tight and constructed in such a manner that any one of its four sides can be easily removed and replaced when necessary. This is essential in handling the heavy blocks in order to obviate the necessity of lifting them high above the ground. For this purpose one side of the tank is removed and the latter is pushed under the block, which is suspended about 20 inches above the ground. As soon as the block is placed on the bottom of the tank, the side is replaced and the tank filled with water. The soaking lasts for several hours, larger blocks receiving more time than smaller ones. The washing is done with the greatest possible care by an experienced person. The stream of water is such as to be sufficiently

gentle for the minute root structures and yet strong enough to loosen and carry away the soil particles. A fan-shaped nozzle with small holes breaking the water stream from an ordinary one-inch garden hose into numerous fine jets seems to comply with both requirements exceptionally well. The strength and volume of the water stream is controlled by a hand operated valve conveniently located near the nozzle (see Fig. 4).

To determine such matters as (a) the greatest depth of penetration, (b) the lateral spread of the main roots, (c) the greatest depth at which side branches of the first order appear on the main roots, (d) the depth at which these produce branches of the second order, etc., the washing is started at the bottom of the block and proceeds upwards until the root system is liberated from the last speck of soil (see Fig. 6).

FIG. 6. *Method of washing soil blocks. Work begins at the bottom of the block so that the depth of penetration and lateral spread of the main roots and side branches may be precisely determined.*

10. Field Records

A report is kept for each block. This consists of two sheets. At the top of the first sheet the name of a plant or plants (in competition) studied is given as a general title. Then records concerning the dates of seeding, emergence and excavation are entered. In addition brief notes on the soil profile, moisture conditions at different depths and the most characteristic features of the root systems are included. On graph paper a pencil chart of the root system is made to scale. On this chart the exact position, depth of penetration, and lateral spread of each main root are indicated not only by location on the chart, but more precisely by numbers inserted in squares at the bottom of the sheet.

11. Preserving the Root System in Fresh Condition for Detailed Analysis

The charts mentioned in the preceding paragraph are very accurate. Each main root is carefully charted from the moment when its tip first appears at the bottom of a block until it joins the underground stem. Nevertheless, there are in each root system many very important details that cannot be recorded at the time of washing. These, however, can be clearly observed and accurately studied if the root system is placed in clear water in a suitable tank. Since time is not available in summer for the analysis of each root system as soon as it is washed, the root material is preserved in a fresh condition until winter for detailed analysis. The material is preserved in a 3 to 4% solution of formaldehyde. The younger plants are kept in glass jars and the large root systems in tanks, each labelled to correspond with the proper field report. With this simple treatment the root systems stored for several months look as natural and fresh as on the day of excavation.

12. Analysis

The analysis is done in special steel or wooden tanks. These are of such size as to receive an entire plant with both top growth and root system spread into their normal positions. In practice, tanks from 8 to 12 feet long, 4 feet wide and 8 inches deep, have proved to be satisfactory for most of the field crops and weeds. The tanks are painted black inside to give the greatest possible contrast between the background and white, or nearly white, root material. At one end each tank has a drain. At the left hand side of the bottom a white scale with one-inch and five-inch divisions is made to indicate the height of the top growth and the depth of each main root. A zero mark corresponding to the ground level is inserted on the scale. From this point on the scale the divisions are numbered upwards and downwards. Another white scale at right angles to the first is made to indicate the lateral spread

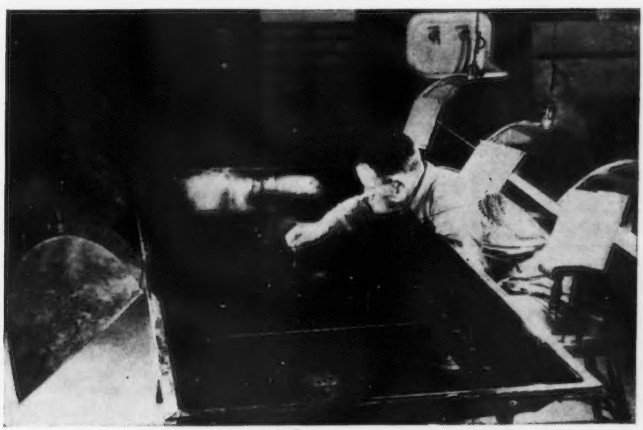

FIG. 7. *The analyzing tank and facilities for illumination.*

of each root. For quick measurement of the root branches the whole portion of the tank under the cross scale may be conveniently divided with white lines into one-inch squares. The tanks are each illuminated with eight 250 w. inside-frosted lamps equipped with special lamp shades which throw the light at the required angle. These are shown in Fig. 7.

Prior to analysis the tanks are thoroughly washed and filled with tap water to a depth of from three to six inches depending on the size of the specimen to be analyzed. The preserved root material is washed free of the formaldehyde solution in cool water and immediately placed in the tank. Weights of convenient shape, made of Babbitt metal, are used to fix the top growth under water, so that the demarcation point between the above and the underground organs falls in line with the cross scale. The main roots are moved into their proper positions as indicated by the original field reports, and fixed there with the weights. If the material represents the root system of a single plant grown free from competition it is ready for detailed analysis. If it consists of several rows of different species competing with one another and each containing many plants, these may be carefully separated and several representative root systems of each kind may be studied. Forms B and C indicate the details of the information collected during these analyses.

FORM B. (GRASS SPECIES)

Project..... Development of the root system of.....*(Name of the crop.)*.....
Section..... grown in 6 inch drill rows in competition with...*(Name of the weed)*.....
Table..... occupying the interspaces between the cereal rows and excavated at .....*(Stage of development)*.....after emergence.

WEED RESEARCH NURSERY 1936

Characters studied	Five plants studied					Totals	Averages
	1	2	3	4	5		
Seminal root system							
No. of seminal roots							
Longest root, inch							
Shortest root, inch							
Max. penetration, inch							
Greatest spread, inch							
Length less br., inch							
Branches, First Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch. per plant							
Length per plant, inch							
Branches, Second Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch. per plant							
Length per plant, inch							

FORM B. (GRASS SPECIES)—*(Continued)*

Characters studied	Five plants studied					Totals	Averages
	1	2	3	4	5		
Branches, Third Order							
Max. No. per lin. inch.							
Min. No. per lin. inch							
Longest branch, inch							
No. branch, per plant							
Length per plant, inch							
Crown Root System							
No. of crown roots							
Longest root, inch							
Shortest root, inch							
Max. penetration, inch							
Max. spread, inch							
Length, less br., inch							
Branches, First Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch, per plant							
Length per plant, inch							
Branches, Second Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch, per plant							
Length per plant, inch							
Branches, Third Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch, per plant							
Length per plant, inch							
Total length of root system per plant, inch							

FORM C. (DICOTYLEDONOUS SPECIES)

Project..... Development of root system of..... *(Name of the weed)*.....
Section..... grown between 6 inch drill rows of..... *(Name of the crop)*.....
Table..... and excavated at..... *(Stage of development)*.....days after emergence.

WEED RESEARCH NURSERY 1936

Characters studied	Five plants studied					Totals	Averages
	1	2	3	4	5		
Tap root							
Greatest depth, inch							
Length less br., inch							
Branches, First Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. br. per plant							
Length per plant, inch							
Branches, Second Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch per plant							
Length per plant, inch							
Branches, Third Order							
Max. No. per lin. inch							
Min. No. per lin. inch							
Longest branch, inch							
No. branch per plant							
Length per plant, inch							
Branches, Fourth Order							
Remarks:							
Total length of root system per plant, inch							

Fig. 8 illustrates the root system of a single wild oat plant photographed in water at the time of analysis. Each of its numerous roots with millions of their branches float freely in the water and can be measured and counted. It is only because of this means of actually seeing, counting, and measuring these structures that it became possible to determine the total length of one root system as being approximately 54 miles. Still more details in the root material as disclosed by this method are demonstrated in Fig. 9. This represents only one crown root separated from the root system referred to in Fig. 8. It shows the crown root penetrating to a depth of 63 inches with its 676 branches of the first and 145,000 of the second order. Detailed results are given in Table I.

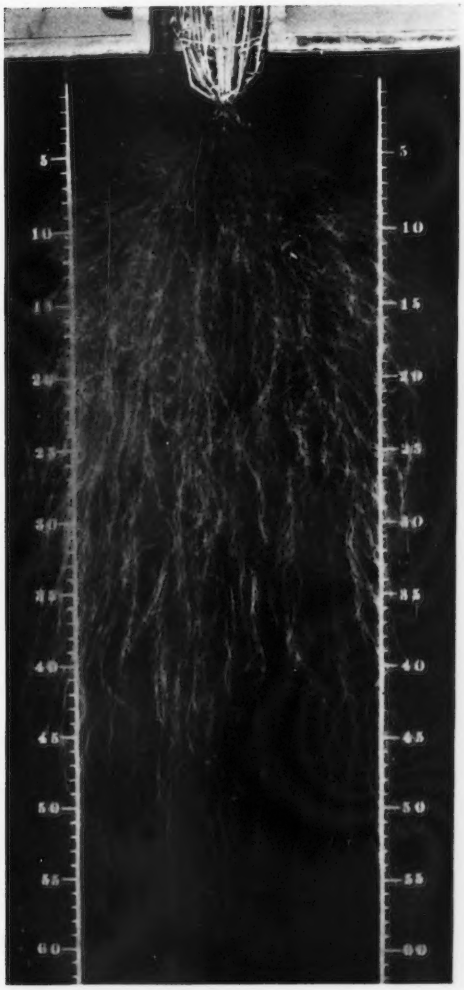

FIG. 8. *The root system of a single wild oat plant, grown free from competition and excavated 80 days after emergence. Total length of roots 54 miles.*

FIG. 9. *One crown root from the root system shown in Fig. 8. Total length of this crown root, 4.05 miles.*

TABLE I

DETAILED STUDY OF ONE CROWN ROOT SEPARATED FROM THE ROOT SYSTEM OF A SINGLE PLANT OF WILD OATS GROWN FREE FROM COMPETITION IN THE CENTRE OF AN AREA TEN FEET SQUARE

Characters studied	Data	Characters studied	Data
Length of the root	63 in.		
Branches of first order		*Branches of first order*—Concluded	
Longest branch	51 in.	Total number of branches of first order	676*
Frequency of branches at different depths:			
0 to 5 in.	9	Total length of branches of first order	19675**
6 to 10 in.	13		
11 to 15 in.	17		
16 to 20 in.	16		
21 to 25 in.	11		
26 to 30 in.	11.6		
31 to 35 in.	10	*Branches of the second order*	
36 to 40 in.	12		
41 to 45 in.	10.4	Longest branch	13 in.
46 to 50 in.	10.2		
51 to 55 in.	11	Average frequency per linear inch	13
56 to 60 in.	11		
61 to 65 in.	0	Greatest depth at which they occurred	48 in.
Average length of branches of first order at different depths:			
0 to 5 in.	34 in.	Their total number	145500†
5 to 10 in.	41 in.		
11 to 15 in.	49 in.	Their total length	237000††
16 to 20 in.	46 in.		
21 to 25 in.	40 in.	Total number of first and second order branches	146176
26 to 30 in.	36 in.		
31 to 35 in.	27 in.		
36 to 40 in.	18 in.	Their total length	256675 in.
41 to 45 in.	10 in.		
46 to 50 in.	4 in.		
51 to 55 in.	2 in.		
56 to 60 in.	0.75 in.		
61 to 65 in.	0.0 in.		

NOTE.—*Branches of third and fourth orders were very numerous but not estimated.*

**These were actually counted.*

***The value represents the sum of results obtained by multiplying the actual number of root branches at each depth by their average lengths.*

†*This number was obtained on the basis of the length of branches of the first order actually bearing branches of the second order, multiplied by the average frequencies of the latter as determined from 250 random counts at each five-inch depth.*

††*The length of branches of the second order was arrived at in the following manner: starting from the surface downward, a hundred random counts and measurements were made at each consecutive five inches in depth, to determine the average frequency and length of the branches. The results thus obtained are summarized in the value indicated.*

A study of the results presented in Table I shows that a multitude of root structures, many of which measure one twenty-fifth of a millimeter in diameter or less, may stretch over four feet in length through hard and frequently very coarse ground. By the use of the soil-block washing method in a joint study by the Dominion Forage Crops laboratory and the Weed Research Nursery

at Saskatoon, it was determined that the root systems of three-year-old single plants of slender wheat grass, brome grass and crested wheat grass measured 9.9, 65.2 and 315.4 miles respectively. Needless to say, the main roots in each case constitute only a negligible fraction of these enormously large quantities. A much greater portion is contributed by the branches of the first order and still more by those of the higher orders. This serves to indicate the importance of the youngest and finest root organs in the formation of the total absorption root surface, which determines the competitive efficiencies of plants. It is this most delicate root material that is most difficult to extricate from the ground and this complicates the problem of root study. However, the soil-block washing method seems to handle this task within the limits of practical needs, and from this point of view it has proved to be a valuable tool in determining the biological merits of economically important plants.

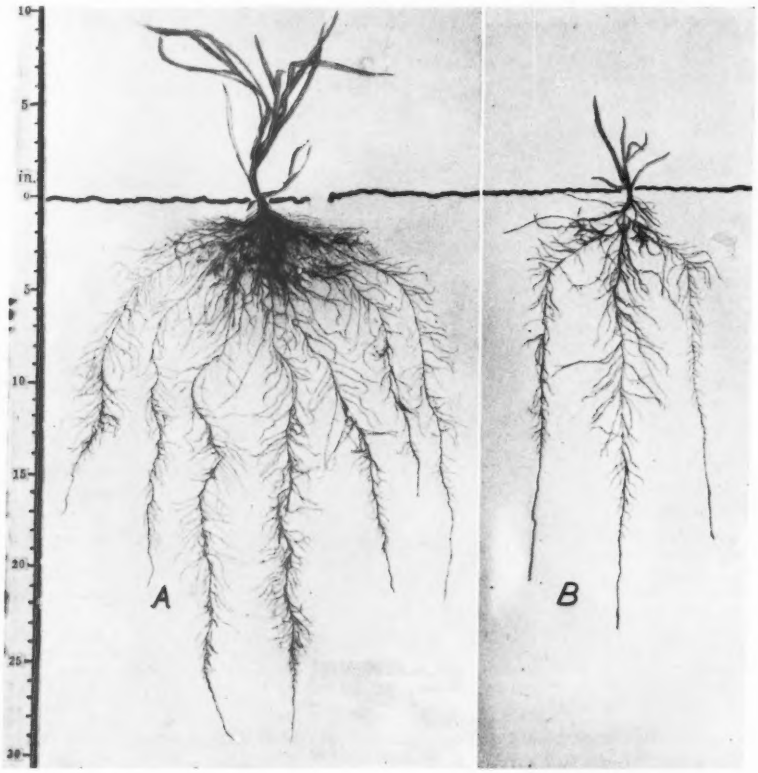

FIG. 10. *Root systems of Hannchen barley (A) and wild oat (B) plants which grew side by side three inches apart. The illustration indicates size and distribution of the root systems. An indication of their interrelations in the soil would be obtained if B were superimposed on A, with the stems three inches apart. These root systems are so arranged on the original mount, from which the photographs were made in water. Twenty-two-day-old material.*

13. Photographing the Root Systems after Analysis

It has already been mentioned that, for the analysis, each root system is properly spread in the water. Since the roots of most species are white or nearly white in color, it is easy to obtain sufficient contrast between them and the deep black bottom of the analyzing tank to photograph them right in the water, where they float freely and assume the most natural forms and positions (see Figs. 8, 9 and 10).

14. Bleaching Process for Dark Root Material

The root material of some species is so dark in color that there is not sufficient contrast between the roots and either a black or white background in the analyzing tank. It is therefore difficult to analyze such specimens, and not possible to photograph or mount them. Brome grass is typical of this class of material. The difficulty was overcome, however, by applying a bleaching process to reduce the dark pigment without impairing the natural strength of the roots. The process is as follows.

(1) Soak roots in a mixture composed of 99 parts of the commercial preparation known as Javelle water, one part of 30% hydrogen peroxide and traces of chlorine water. Prepare sufficient of the mixture to cover the root material completely. The latter should remain in the mixture until the dark pigmentation disappears.

(2) Wash the roots in clean soft water.

(3) Bathe them for 10 minutes in thick suds of sodium oleate.

(4) Wash very thoroughly in soft water, and drain.

(5) Dip the roots in 95% alcohol for two minutes, drain and wash with soft water.

15. Mounting of Root Specimens

Mounting also is best done under water. In this case a suitable mounting background, prepared ahead of time, is slowly pushed under the root system. By careful manipulation of long needles, the main roots and their branches are moved into their proper places on the background and fixed by the Babbitt weights. As soon as this is done the drain cock is half opened and in a short time the root system is left in the natural shape on the mounting background. In mounting the root systems of unrelated species obtained from competition plots it is possible to dye these in different colors, spread them under water so as to represent their interrelation in the ground and finish the mounting as in the previous case.

This method of mounting requires a good deal of experience, but it certainly gives a splendid opportunity of demonstrating the nature, extent and distribution of root systems in a most satisfactory form. Employing this technique a very extensive collection of root systems of various cereal and forage crops and weed species has been established at the University of Saskatchewan. This collection may be regarded as the beginning of a root study museum.

Acknowledgments

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References

1. ARKER, J. Die Beeinflussung des Wachsthums der Wurzeln durch das umgebende Medium. Botan. Centr. 87 : 433-434. 1901.
2. COTTRELL, H. M. Growing alfalfa in Kansas. Kansas Agr. Expt. Sta. Bull. 114 : 61-62. 1902.
3. FRANK, B. Die Assimilation des freien Stickstoffs durch die Pflanzenwelt. Botan. Ztg. 51 : 139-156. 1893.
4. GOFF, E. S. Report of the Horticulturist. New York Experimental Station. Year 1883, p. 219. Year 1884, pp. 305-315. Year 1886, pp. 159-168. Year 1887, pp. 90-96. Year 1888, pp. 171-178.
5. GOFF, E. S. A study of the roots of certain perennial plants. Wis. Agr. Expt. Sta. 14th. Ann. Rep. pp. 286-298. 1897.
6. HALES, S. Vegetable staticks. pp. 4-7. 1727.
7. HAYS, W. M. Corn, its habit of root growth, methods of planting and cultivating, notes on ears and stools or suckers. Minn. Agr. Expt. Sta. Bull. 5 : 5-33. 1889.
8. HEADDEN, W. P. Alfalfa. Colo. Agr. Expt. Sta. Bull. 35 : 47-58. 1896.
9. HELLRIEGEL, H. Wurzel und Bodenvolumen. Beitr. Naturw. Grundl. Ackerbaus, 119-280. 1883.
10. HOLBERT, J. R. and KOEHLER, B. Anchorage and extent of corn root systems. J. Agr. Research, 27: 71-78. 1924.
11. HOVELER, W. Uber die Verwertung des Humus bei der Ernaehrung der chlorophyll-fuehrenden Pflanzen. Jahrb. wiss. Botan. 24 : 283-316. 1892.
12. JEAN, F. C. and WEAVER, J. E. Root behavior and crop yield under irrigation. Carnegie Inst. Washington, Pub. 357. p. 65. 1924.
13. JENSEN, J. H. Some studies of root habits of sugar cane in Cuba. Tropical Plant Research Foundation. Sci. Contr. No. 21. (1086 North Broadway, Yonkers, N.Y.) 1931.
14. KACHINSKI, N. A. Izuchenie fizicheskich Svoistv pochvy i kornievich seest'em rastieniy. (Study of physical properties of soil and the root systems of plants.) Gosizd. Moskva. (State publishing, Moscow, 1931.)
15. KING, F. H. Natural distribution of roots in field soils. Wis. Agr. Expt. Sta. 9th Ann. Rep. pp. 112-120. 1892. 10th Ann. Rept. pp. 160-166. 1893.
16. KRAUS, C. Untersuchungen ueber die Bewurzelung der Kulturpflanzen in physiologischer und kultureller Beziehung. Fortschritte Geb. agr. phys. 15 : 234-286. 1892; 17 : 55-103. 1894; 18 : 113-166. 1895.
17. KRAUS, C. Das Wurzelsystem der Runkelrueben und dessen Beziehung zur Ruebenkultur. Fortschritte Geb. agr. phys. 11 : 358-406. 1888.
18. MASCHHAUPT, J. G. Root systems of agricultural plants. Verslag. Land. Onderzoek. Rijkslandbouwproefst. 16 : 76-89. 1915.
19. MIELECKI, L. Wplyw surowych soli potasowych na rozwoj korzeni roslin. (Effect of salts of potash on development of plant roots.) Doswiadczalnictwo rolnicze. (Agricultural investigation.) 9 : 19-55. 1933.
20. MODESTOV, A. P. Development of roots in the races of the same species. (Title derived from the content.) J. Botan. 35 : 555. 1921.
21. MORROW, G. E. and HUNT, T. F. Field experiments with corn. Ill. Agr. Expt. Sta. Bull. 4 : 113-115. 1889; Bull. 8 : 263-266. 1889; Bull. 13 : 423-428. 1890.
22. NOBBE, F. Ueber die feinere Veraestelung der Pflanzen-wurzeln. Landw. Vers. Stat. 4 : 212-224. 1862.

23. OTUKA, YOSIO. Studies on the beginning period of the growth and the rest period of apple root. Agr. Expt. Sta. South Manchuria Railway Co. (In Chinese, English summary.) Research Bull. 5 : 1-12. 1931.

24. PAVLYCHENKO, T. K. Quantitative study of the entire root systems of weed and crop plants under field conditions. Ecology 18. (1) 1937.

25. PAVLYCHENKO, T. K. and HARRINGTON, J. B. Competitive efficiency of weeds and cereal crops. Can. J. Research, 10 : 77-94. 1934.

26. PAVLYCHENKO, T. K. and HARRINGTON, J. B. Root development of weeds and crops in competition under dry farming. Sci. Agr. 16 : 151-160. 1935.

27. ROTMISTROFF, V. G. Root systems of cultivated plants of one year's growth. Odessa Expt. Sta. Bull. pp. 55. 1909.

28. ROTMISTROFF, W. G. Das Wesen der Dürre ihre Ursache und Verhütung. (The nature of drought, its causes and prevention.) Verlag von Theodor Steinkopff, Dresden und Leipzig. 1926.

29. SCHUBART, ... Erfahrungen und Beobachtungen ueber die Wurzelbildung und Wurzeltiefe mehrerer landwirtschaftlicher Kulturpflanzen, wie ueber die Keimkraft einiger Samen koerner. Chem. Ackersmann, 1 : 193-201. 1855.

30. SCHULZE, B. Studien ueber die Bewurzelung unserer Kulturpflanzen. Festschrift, 50 Jubileum Agr. Chem. Versuchs. und Kontroll Stat. Breslau, 67-95. 1906.

31. SEELHORST, C. von. Beobachtungen ueber die Zahl und den Tiefgang der Wurzeln verschiedener Pflanzen bei verschiedener Duengung des Bodens. J. Landw. 50 : 91-104. 1902.

32. SEELHORST, C. VON und FRECKMANN, W. Der Einfluss des Wassergehaltes des Bodens anf die Ernte und die Ausbildung verschiedener Getreide-Varietaeten. J. Landw. 51 : 253-269. 1903.

33. SHEPPERD, J. H. Root systems of field crops. North Dakota Agr. Expt. Sta. Bull. 64 : 529-535. 1905.

34. SIMMONDS, P. M., RUSSELL, R. C. and SALLANS, R. J. A comparison of different types of root rot of wheat by means of root excavation studies. Sci. Agr. 15 : 680-700. 1935.

35. SPIRHANZL, J. Pokus o zjistovani hloubky a zpusobu zakorenovani plodin. (Study on depth and mode of penetration of plant roots.) Sbornik Ceskoslovenske Akademie Zemedelske. 11 : 298-303. 1936.

36. TEN EYCK, A. M. A study of the root systems of cultivated plants grown as farm crops. North Dakota Agr. Expt. Sta. Bull. 43 : 535-548. 1900.

37. TUCKER, M. and SEELHORST, C. von. Der Einfluss der Wassergehalt und der Reichtum des Bodens auf die Ausbildung der Wurzeln und der oberirdischen Organe der Haferpflanze ansueben. J. Landw. 46 : 53-63. 1898.

38. WEAVER, J. E. The ecological relations of roots. Carnegie Inst. Washington, Pub. 286 : 2-3. 1919.

39. WEAVER, J. E. and CRIST, J. W. Relation of Hardpan to root penetration in the Great Plains. Ecology, 3 : 237-249. 1922.

THE SYNTHETIC PRODUCTION OF OAT VARIETIES RESISTANT TO RACE 6 AND CERTAIN OTHER PHYSIOLOGIC RACES OF OAT STEM RUST[1]

By J. N. Welsh[2]

Abstract

At the present time, oat varieties that are classed as resistant to *Puccinia graminis Avenae* Erikss. & Henn. are only resistant to a certain number of the ten physiologic races. With the object of combining in a single variety resistance to as many races as possible, a cross was made between the varieties Hajira Strain and Joanette Strain. Hajira Strain is susceptible to Races 4, 6, 8, and 10, and Joanette Strain to Races 2, 6, 7, 8, and 9. The latter variety gives an indeterminate reaction to Races 5 and 10. Both parents are susceptible to Races 6 and 8.

From this cross 93 pure lines were obtained. Under greenhouse conditions, 71 were resistant at the seedling stage to Race 6 at 60° F. At 65°–70° F., approximately one-third of these were resistant to Race 6, one-third semi-resistant, and one-third susceptible. At more advanced stages of growth, namely, fifth-leaf, boot, and heading, representative lines from each of these classes were resistant to Race 6 at 60° F. At 65°–70° F. all showed regional resistance: at the fifth-leaf stage, the tip end of the uppermost leaf only was susceptible; at the boot stage, numerous pustules were present on the uppermost node and internode but the remaining parts were free from infection; at the heading stage, only one or two fairly large pustules occurred on the uppermost node or internode.

Six lines that were consistently resistant to Race 6 at 60° F. and 65°–70° F. were tested at the seedling stage at 60°, 65°–70° F., and 75°–80° F., to Races 1, 2, 3, 4, 5, 6, 7, 8, and 10. At the low and intermediate temperatures, these lines were resistant to the nine races. At the high temperature, they were susceptible to Race 6, gave an indeterminate reaction to Races 1, 4, and 5, and were resistant to all the other races.

Under field conditions, six lines classed as resistant at 65°–70° F., five classed as semi-resistant, and four as susceptible, were tested to Race 6. All these lines behaved similarly: infections of a semi-resistant type appeared on the uppermost internodes, while other parts of the plants were free from infection.

The standard varieties used as checks, namely, Hajira Strain, Joanette Strain, White Russian, and Victory, were susceptible to Race 6 in all the greenhouse experiments, and, with the exception of White Russian, in the field test. In the latter test, White Russian was semi-resistant.

Introduction

One of the many problems confronting the plant breeder is to combine in a single variety of oats resistance to all the physiologic races of *Puccinia graminis Avenae* Erikss. & Henn. Ten races (previously called forms) of oat stem rust are known at the present time and no variety is known to be resistant to all of them. The varieties classed as resistant to oat stem rust differ in their reactions to some of these races. Some are resistant to certain ones and susceptible to others; none are resistant to Race 6.

These varieties may be classified into three groups according to their seedling reaction to the several races. The first group is represented by Hajira Strain, which is resistant to Races 1, 2, 3, 5, and 7 and semi-resistant to Race 9;

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Contribution from the Cereal Division, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

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the second by White Russian, which is semi-resistant to Races 1, 2, 5, 8, 9, and 10; and the third, by Joanette Strain which is resistant to Races 1, 3, 4, and 10 and gives an indeterminate reaction to Race 5.

The infection types produced by the races to which Hajira Strain and White Russian are resistant are not affected by changes of temperature (See Table IV). The types of those to which Joanette Strain is resistant, on the other hand, are affected by such changes. Waterhouse (6) found that this variety was resistant to Race 1 at low temperatures, but completely susceptible to it at high temperatures. Gordon (1, 2) showed that Joanette Strain was susceptible, both at the seedling and mature stages, to Races 1, 3, 4, and 5 at 75.4° F., but was resistant to Races 1, 3, and 4, and produced an indeterminate reaction to Race 5 at 57.4° F. Newton and Johnson* found that Joanette Strain was resistant to Race 10 at 60° F., susceptible at 75°–80° F., and gave an indeterminate type of reaction at 65°–70° F.

Up to the present, not a great deal has been accomplished in the building up of resistance to the several physiologic races of oat stem rust. Smith (5) found that, in a Gopher × Rainbow cross, lines resistant or susceptible to Races 1, 2, 3, 5, and 7 reacted similarly to Race 8, but that lines segregating for resistance to this group of five races also segregated for resistance to Race 8. In a Hajira Strain × Joanette Strain cross (hereinafter referred to as Hajira and Joanette) Welsh (7) combined the resistance of these two varieties and obtained lines resistant to Races 1, 2, 3, 4, 5, and 7.

The present study deals with the reaction of pure line selections of this cross to nine of the ten races. Race 9 was not available for the study. These lines were inoculated in the greenhouse with the nine races at the seedling stage and with Race 6 at the more advanced stages of growth, at different temperatures. Some of the selections were also inoculated with Race 6 under field conditions. The results of these tests are presented in this paper.

Greenhouse Reactions to Race 6

Three F_3 lines of the Hajira × Joanette cross were inoculated with Race 6. A number of lines from this cross were previously shown (7) to be resistant to Race 4, a race to which Joanette is resistant, and to Races 1, 2, 3, 5, and 7, to which Hajira is resistant. To check further the resistance of these lines to Race 4, they were again inoculated with this race. Three lines that appeared particularly resistant to Race 4 in this test were inoculated with Race 6. One of the three lines was homozygous for resistance to this race, while the other two segregated. All of the resistant seedlings were transplanted and grown to maturity in the greenhouse. The progeny of these were then inoculated with Race 6 and again the most resistant seedlings were selected and transplanted. This procedure was continued until the plants had reached the sixth generation, by which time 93 pure lines were obtained.

* *Unpublished data, Dominion Rust Research Laboratory, Winnipeg, Canada.*

It was observed throughout these infection tests that a number of the seedlings gave an indeterminate type of reaction. It was also noted that the reactions, in general, were heavier when the temperature in the greenhouse was comparatively high.

These 93 lines were inoculated with Race 6 in January, during which month there was very little sunshine. The greenhouse was kept at 60°–65° F., owing to the presence of another experiment requiring that temperature. Of the 93 lines inoculated, 71 were resistant, 6 semi-resistant, 4 susceptible, and 12 gave an indeterminate type of reaction. In February, and again in

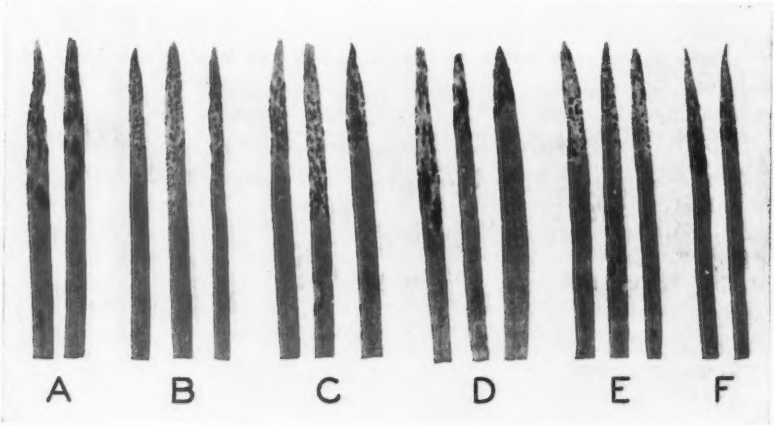

FIG. 1. *Seedling reaction classes, at 65°–70° F., on lines of a Hajira × Joanette cross, inoculated with Race 6.* A *and* F,—*(Hajira and Joanette respectively), susceptible;* B,—*resistant;* C,—*semi-resistant;* D,—*susceptible;* E,—*indeterminate.*

March, the 71 resistant lines were further tested to Race 6. There was practically continuous sunshine during the day throughout these two months and the temperature in the greenhouse was approximately 65°–70° F., although sometimes higher. Under these conditions the infections were heavier and more of the lines gave the indeterminate reaction than in the January test. The lines were classified according to their reactions into four groups: resistant, semi-resistant, indeterminate, and susceptible. The reaction classes observed during the two latter tests are shown in Fig. 1. As the lines reacted differently at the low and intermediate temperatures, experiments were planned to test the reaction of the lines under conditions of controlled temperature.

Effect of Temperature on the Reactions of Lines to Race 6 at Different Stages of Growth

In order to determine more definitely the effect of temperature on the reaction of the lines to Race 6, certain lines were tested at different temperatures at the seedling and more advanced stages of growth, namely, fifth-leaf, boot and heading.

Reactions at Seedling Stage

Twenty-four lines (eight from each of the lines formerly classified at 65°–70° F. as resistant, semi-resistant, and susceptible) were tested at 60° F. and 65°–70° F. Six of the eight resistant lines were tested at 75°–80° F. The standard varieties used as checks in this and subsequent tests were Hajira, Joanette, White Russian, and Victory.

At 60° F., all of the 24 lines were resistant. At 65°–70° F., the eight lines classed as resistant remained resistant and those of the semi-resistant and

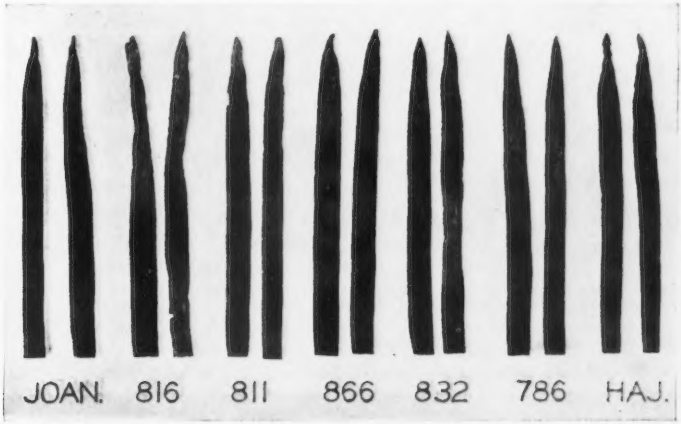

FIG. 2. *Seedling reactions, at 60° F., of parents and lines of a Hajira × Joanette cross to Race 6.*

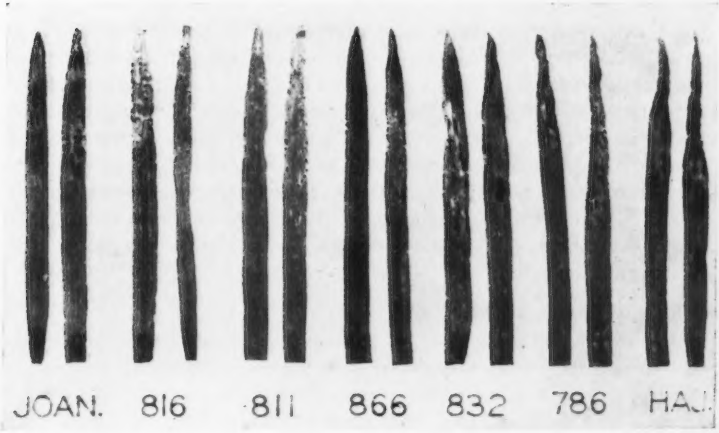

FIG. 3. *Seedling reactions, at 65°–70° F., of parents and lines of a Hajira × Joanette cross to Race 6.*

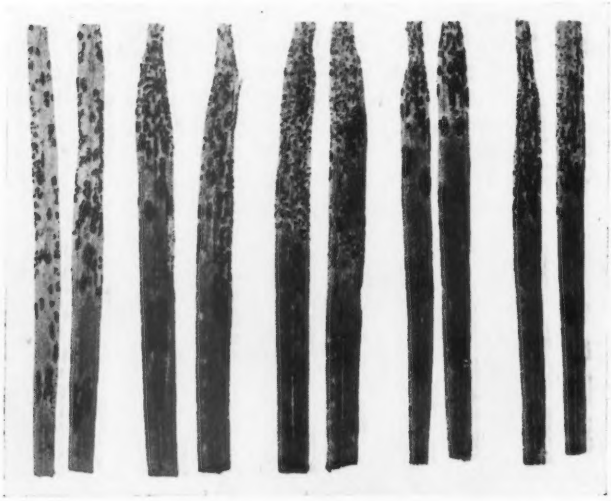

FIG. 4. *Seedling reactions, at 75°–80° F., of the four standard varieties used as checks and one line of a Hajira × Joanette cross, to Race 6. Left to right: Line 811, Victory, White Russian, Joanette, and Hajira.*

susceptible classes again gave semi-resistant and susceptible reactions, respectively, thus confirming the former observations that at intermediate temperatures some lines become susceptible, while others remain resistant. At 75°–80° F., the six resistant lines tested were completely susceptible. The results show definitely that in the seedling stage the reactions of these lines to Race 6 are affected by temperature changes.

The reactions at the three temperatures are shown in Figs. 2, 3, and 4. Figs. 2 and 3 show the reactions at 60° F. and 65°–70° F., respectively, of two of the resistant lines (Nos. 816 and 811), one of the semi-resistant (No. 866), and two of the susceptible ones (Nos. 832 and 786), together with the reactions of the two parents. It will be observed that lines 832 and 786 gave a resistant reaction at the low temperature and a susceptible one at the higher temperature, while the reactions of the other lines showed little or no change. Fig. 4 shows the reaction at 75°–80° F. of one of the lines (No. 811) that was resistant at the two lower temperatures and the reaction of the four standard varieties.

Reactions at More Advanced Stages

As the reactions of the lines at the seedling stage were affected by temperature, an experiment was planned to determine whether the lines would show similar behavior at other stages of growth, namely, fifth-leaf, boot, and heading. Six lines, two from each of the groups previously classified at 65°-70° F. as resistant, semi-resistant, and susceptible, and the four standard varieties were inoculated with Race 6 in the above-mentioned stages. The

tests were conducted in duplicate at two temperatures 60° and 65°–70° F. Owing to the extremely cold weather that prevailed at the time the tests were conducted, temperatures higher than 70° were not obtainable. The lower temperature varied relatively little, but the higher temperature frequently dropped as low as 60° F. at night.

At 60° F., the six lines just referred to were resistant at all stages. At 65°–70° F., however, the reactions differed somewhat at the different stages, but all the lines reacted similarly at a given stage. At the fifth-leaf stage, all culms and leaves, with the exception of the uppermost leaf, were resistant. A portion of this leaf, from one to three inches back from the tip, was susceptibe. At the boot stage, the leaves were resistant, whereas the culms gave a regional type of resistance in which the infections occurred only on the upper node and internode of each plant. At the heading stage, the leaves and culms were quite resistant, although generally one or two fairly large pustules could be found on the uppermost internodes. The standard varieties were susceptible at all stages at both temperatures. Temperature, therefore, affects the reaction of the lines at the more advanced stages of growth as well as in the seedling stage.

Field Reactions to Race 6

A field experiment was conducted in 1936 with six of the resistant, five of the semi-resistant, and four of the susceptible lines (so classed at 65°–70° F.)

TABLE I

FIELD AND GREENHOUSE REACTIONS OF STANDARD VARIETIES AND LINES OF A HAJIRA × JOANETTE CROSS INOCULATED WITH RACE 6

Hybrid lines and varieties	Seedling reaction (65°–70° F.)	Field reaction			
		Range of pustule types	Rust percentages		
			Replicate 1	Replicate 2	Average
792	SR	3 – 3+	25	15	20
791	SR	3 – 3+	30	20	25
807	S	3 – 3+	40	20	30
811	R	3 – 3+	20	15	18
790	R	3 – 3+	15	30	23
806	S	3 – 3+	20	40	30
835	S	3 – 3+	25	15	20
863	SR	3 – 3+	15	25	20
814	SR	3 – 3+	15	30	23
839	S	3 – 3+	30	40	35
780	R	3 – 3+	15	25	20
846	R	3 – 3+	25	25	25
793	R	3 – 3+	35	35	35
862	SR	3 – 3+	30	30	30
794	R	3 – 3+	30	35	33
Hajira	S	4	40	35	38
Joanette	S	4	55	55	55
White Russian	S	2 – 3	25	30	28
Victory	S	4	35	55	45

Greenhouse reaction (seedling): R = *resistant;* SR = *semi-resistant;* S = *susceptible.*
Field reaction (Pustule type): 2 = *resistant;* 3 = *semi-resistant;* 4 = *susceptible.*

to study their resistance to Race 6 under field conditions and to determine the relation between the field and the seedling reactions. The four standard varieties were used as checks. The seed was planted in single rod rows, in duplicate. Two guard rows of Victory were planted around the experimental plot. Inoculum of Race 6 was provided by transplanting heavily infected plants between the guard rows and also by injecting inoculum into the tissues of the plants in the guard rows on two occasions. When the plants were mature, rust percentage and pustule type were recorded. The data are summarized in Table I.

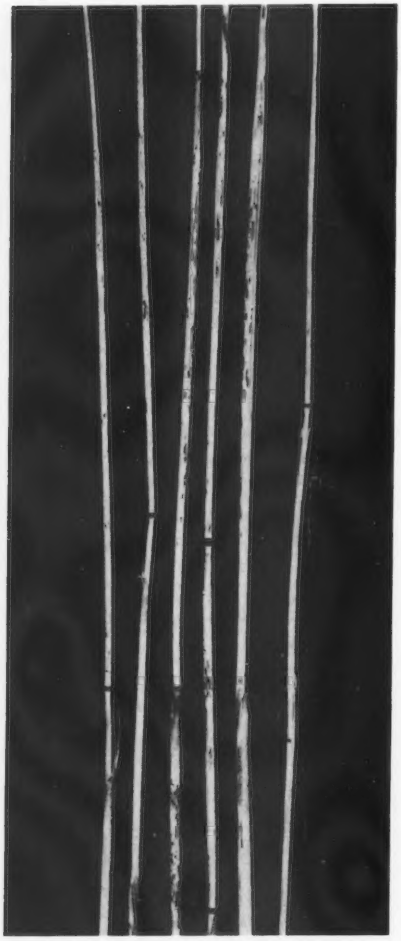

FIG. 5. *Field reactions to Race 6 of the four standard varieties used as checks and two lines of a Hajira × Joanette cross. Left to right: Line 811, Line 793, Hajira, Joanette, Victory and White Russian.*

The lines gave a regional type of reaction, in which the infections occurred only on the uppermost internodes, a reaction similar to that produced in the greenhouse by the lines in the boot stage. The standard varieties, on the other hand, were rusted throughout the entire length of the culm. The rust percentages on all plants were based on comparable areas, namely, on the most heavily rusted six inches, and therefore do not represent the amount of rust present on the whole plant. On this account only slight differences in reaction between the lines and varieties appear in Table I. The average infection on the lines ranged from 18 to 35% and that on the standard varieties from 28 to 55%. The pustule types produced on the lines ranged from 3 to 3+, while those on the standard varieties, with the exception of White Russian, which gave a 2 to 3, were of the 4 type. White Russian had only 28% infection and produced a 2 to 3 type of pustule. This reaction is difficult to explain, as this variety is quite susceptible to Race 6 in the greenhouse, at all stages of growth. The reactions of two of the lines and the four standard varieties are shown in Fig. 5.

The data were analyzed statistically to determine whether the differences in reaction (i) between the lines and varieties, and (ii) within or between the resistant, semi-resistant, and susceptible classes, were significant. The results of the analysis are given in Table II.

TABLE II

ANALYSIS OF RUST PERCENTAGES ON STANDARD VARIETIES AND LINES OF A HAJIRA × JOANETTE CROSS INOCULATED WITH RACE 6 UNDER FIELD CONDITIONS

Variance due to	Sums of squares	D.F.	Variance	F	5% point
Within resistant lines	485.4	5	97.1	1.57	2.77
Within semi-resistant lines	140.0	4	35.1	—	—
Within susceptible lines	237.5	3	79.2	1.29	3.16
Between groups of lines	123.8	2	61.4	—	—
Standard varieties	812.5	3	270.8	4.40	3.16
Lines *vs.* varieties	1533.7	1	1533.7	24.90	4.41
Replicates	65.8	1	65.8	1.07	4.41
Error	1109.2	18	61.6		
Total	4507.9	37			

The data in Table II show that no significant differences in reaction were obtained between lines within the resistant, semi-resistant or susceptible class, or between these three classes. It is evident, then, that as far as field resistance is concerned, all these lines react in a similar manner, regardless of their classification as resistant, semi-resistant, or susceptible at 65°–70° F. in the greenhouse. As the lines were all resistant at 60° F. their resistance at this temperature, therefore, is a criterion of their resistance under field conditions. To show further the lack of agreement between the greenhouse and the field reactions the data were arranged in a 2 × 2 table. The field rust percentages were classified on a resistant, semi-resistant, and susceptible basis to correspond with the greenhouse classifications. The class range for each group was 5%. The data are given in Table III.

TABLE III

RELATION BETWEEN THE RUST REACTION TO RACE 6 OF THE LINES OF A HAJIRA × JOANETTE CROSS IN THE GREENHOUSE AT 65°–70° F. AND UNDER FIELD CONDITIONS

Field \ Greenhouse	R	SR : S	
R	3	4	7
SR : S	3	5	8
	6	9	15

The data in this table show that there is no definite relation between the field reaction and the greenhouse reaction at 65°–70° F. Significant differences were obtained, however, within standard varieties, and between the varieties

and lines. The fact that White Russian was more resistant than the other standard varieties accounts for the differences in reaction between the varieties. As the reaction of the lines and varieties differed significantly, it can be concluded that the lines are more resistant than the standard varieties.

Greenhouse Reactions to Other Physiologic Races at Different Temperatures

Six of the lines that were consistently resistant to Race 6 at the low and intermediate temperatures were inoculated at the seedling stage with other races at ordinary greenhouse temperatures (65°–70° F.) on three different occasions. As a further check on the resistance of these lines to Race 6, this race also was included in these tests. In the first two tests the lines were inoculated with Races, 1, 2, 3, 4, 5, 6, 7, and 8, and proved resistant to all these races in both tests. In the third test Race 10 was included. The lines were resistant to this race also.

As the lines were resistant to Race 6 at the low and intermediate temperature, but susceptible to it at the high temperature, an experiment was planned to determine the reaction of the lines to the other races at 60°, 65°–70°, and 75°–80° F. Six of the most resistant lines, together with the four standard varieties, were inoculated with Races 1, 2, 3, 4, 5, 6, 7, 8, and 10 at these three temperatures. The six lines reacted similarly. The reactions of one of the lines, namely, No. 811, and of the four standard varieties are given in Table IV.

It will be seen in Table IV that this line (No. 811), representative of the other five lines, is not only resistant to the nine races at the low and intermediate temperatures but possesses, in general, a higher resistance than the standard varieties possess at these two temperatures to the races to which they are resistant. At 60° F., Line 811 gives a very resistant reaction to eight of the nine races and a resistant reaction to Race 6. At 65°–70° F., this line is resistant to Races 3 and 6, and very resistant to the other races. At 75°–80° F., it is resistant to Races 2, 3, 7, 8 and 10, gives an indeterminate type of reaction to Races 1, 4, and 5, and a susceptible reaction to Race 6.

Discussion of Results

The problem of developing oat varieties resistant to all physiologic races of *Puccinia graminis Avenae* is an important one as the resistant varieties which are at present grown commercially, or used for plant breeding purposes, possess resistance to only a certain number of the races. None of these varieties is resistant to Race 6. The resistance possessed by these varieties is effective only when the races present are those to which the variety or varieties in question are resistant. As races to which these varieties are susceptible sometimes appear in the field, it is desirable, therefore, to obtain oat varieties resistant to all races. An advance towards that objective is reported in this paper.

TABLE IV

THE REACTIONS OF THE STANDARD VARIETIES AND ONE LINE OF A HAJIRA × JOANETTE CROSS TO NINE PHYSIOLOGIC RACES OF STEM RUST AT LOW, INTERMEDIATE, AND HIGH TEMPERATURES—60° F., 65°–70° F., AND 75°–80° F., RESPECTIVELY

Varieties	Physiologic races																										
	1			2			3			4			5			6			7			8			10		
	Temp.			Temp.			Temp.			Temp.			Temp.			Temp.			Temp.			Temp.			Temp.		
	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H
Line 811	VR	VR	I	VR	VR	R	VR	R	R	VR	VR	I	VR	VR	I	R	R	S	VR	VR	R	VR	VR	R	VR	VR	R
Joanette	VR	I	S	S	S	S	R	I	S	R	I	S	I	I	S	S	S	S	S	S	S	S	S	S	R	I	S
Hajira	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	S	R	R	R	S	S	S	S	S	S
WhiteRussian	SR	SR	SR	SR	SR	SR	S	S	S	S	S	S	SR	SR	SR	S	S	S	S	S	S	SR	SR	SR	SR	SR	SR
Victory	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Temperature classification: L = *low (60° F.);* I = *intermediate (65°–70° F.);* H = *high (75°–80° F.).*

Reaction classification: VR = *very resistant;* SR = *semi-resistant;* I = *indeterminate;* S = *susceptible.*

Resistance to Races 1, 2, 3, 4, 5, 6, 7, 8, and 10 was obtained from a cross between the varieties Hajira and Joanette. Hajira, at all temperatures, is resistant to Races 1, 2, 3, 5, and 7, while Joanette, at low temperatures, only, is resistant to Races 1, 3, 4, and 10, and gives an indeterminate reaction to Race 5. Resistance to Races 6 and 8 was probably obtained through the medium of transgressive segregation, as neither parent is resistant to these two races.

The resistance of the lines at the seedling stage to Races, 1, 4, 5, and 6, and at the more advanced stages of growth to Race 6, was influenced somewhat by temperature. Furthermore, the reactions at the more advanced stages of growth in the greenhouse and at the mature stage in the field were regional in nature. At the seedling stage the lines were resistant to the nine races at the low and intermediate temperatures, but susceptible to Race 6, and gave an indeterminate reaction to Races 1, 4, and 5 at a high temperature. At the more advanced stages the lines were resistant in all stages at 60° F., but at 65°–70° F. they gave a regional type of resistance to Race 6 in which the infections occurred only on certain parts of the plant. At the fifth-leaf stage only the tip end of the uppermost leaf was susceptible, while at the boot, heading, and mature stage in the field only the uppermost nodes and internodes were infected.

As the lines were susceptible to Race 6 at the high temperature and gave an indeterminate reaction to Races 1, 4, and 5, three of the races to which Joanette gives an indeterminate reaction at the intermediate temperature, it is probable that this instability of reaction has been inherited from that parent. Further evidence in support of this hypothesis may be drawn from the fact that the reaction of Hajira, the other parent, to the races to which it is resistant, is not affected by changes of temperature.

The fact that, at the more advanced stages of growth, infections occurred only on certain parts of the plant indicates a regional type of resistance as suggested by Goulden *et al.* (3). These investigators observed that at the adult stage of certain wheat varieties, the region above the nodes and the culms between the uppermost leaf and the head rusted more heavily than other parts of the plants. Furthermore, Newton and Brown (4) have shown that the young rapidly growing parts of resistant varieties of wheat, oats, and barley, when inoculated by injection with a suspension of uredospores shortly before the plants come into head, are very susceptible, while the older, more mature parts are highly resistant. There appears to be considerable similarity between those results and the present findings.

That the infections only appeared at the tip end of the uppermost leaf at the fifth-leaf stage may be explained by assuming that only that portion of the leaf had protruded from the sheath at the time of inoculation, and being in a rapidly growing condition, became infected. On the other hand, this leaf was resistant at the boot stage. In fact, at the boot stage the only susceptible parts were the uppermost nodes and internodes. It is rather difficult to account for the appearance of pustules on these parts, as at the

time of inoculation they were enclosed within the sheath. Most likely the inoculum came into contact with them by entrance through the split in the sheath. These plants, being young and growing rapidly, were susceptible, and hence became infected.

Acknowledgments

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References

1. GORDON, W. L. Effect of temperature on host reactions to physiologic forms of *Puccinia graminis Avenae* Erikss. and Henn. Sci. Agr. 11 : 95-103. 1930.
2. GORDON, W. L. A study of the relation of environment to the development of the uredinial and telial stages of the physiologic forms of *Puccinia graminis Avenae* Erikss. and Henn. Sci. Agr. 14 : 184-237. 1933.
3. GOULDEN, C. H., NEWTON, M. and BROWN, A. M. The reaction of wheat varieties at two stages of maturity to sixteen physiologic forms of *Puccinia graminis Tritici*. Sci. Agr. 11 : 9-25. 1930.
4. NEWTON, M. and BROWN, A. M. Studies on the nature of disease resistance in cereals. I. The reactions to rust of mature and immature tissues. Can. J. Research, 11 : 564-581. 1934.
5. SMITH, D. C. Correlated inheritance in oats of reaction to diseases and other characters. Minn. Agr. Exp. Sta. Tech. Bull. 102. 1934.
6. WATERHOUSE, W. L. Australian rust studies. I. Proc. Linnean Soc. N.S. Wales, 54 : 615-680. 1929.
7. WELSH, J. N. The inheritance of stem rust and smut reaction and lemma colour in oats. Sci. Agr. 12 : 209-242. 1931.

DETERMINATION OF THE DIASTATIC POWER OF MALT IN DEGREES LINTNER BY MEANS OF A FERRICYANIDE REAGENT[1]

By J. Ansel Anderson[2] and Henry R. Sallans[3]

Abstract

It is proposed that the Official Method, of the American Society of Brewing Chemists, for the determination of the diastatic power of malt be modified to permit the use of the Blish and Sandstedt ferricyanide method for determining the reducing power of the digested starch solution. The proposed method involves the use of half the quantity of infusion, rather than twice the quantity of starch, for making diastases of malts with Lintner values of over 135° L. Both changes increase the speed of determination without loss of precision or accuracy. For routine purposes additional speed can be obtained by omitting the dilution of the infusion and by requiring a blank correction for the reducing power of the starch only.

Experimental data show that under the conditions of the determination the ferricyanide method provides an accurate measure of the reducing power of the digested starch solution, and that the results obtained by the two methods, for 16 malts with diastatic powers covering the range from 72° to 185° L., agree to within 3%.

The Official Method of the American Society of Brewing Chemists for the determination of the diastatic power of malt (1, pp. 16–18), which has also been tentatively adopted by the Association of Official Agricultural Chemists (2, pp. 158–160) and which is being studied by the American Association of Cereal Chemists, leaves much to be desired as a routine method. Its unsuitability is amply proved by data given by Coleman (5) which show that 17 laboratories obtained results varying between 103° and 158° L. for the same malt.

These differences between laboratories may result from variations in technique at many stages of the procedure, but undoubtedly are due largely to the use of a titration of boiling Fehling's solution for the determination of the reducing power of the digested starch solution. This titration is notoriously unreliable since it is subject to large personal errors which can be overcome only by close adherence to detailed specifications and by standardizing the Fehling's solution under conditions identical with those used in the determination. These points have hardly received sufficient emphasis in the Official Method.

A second fault which reduces the usefulness of the method is its slowness. This again can be attributed largely to the use of Fehling's solution. Four titrations are required for each determination and additional time is required for rinsing and filling the burettes with different solutions.

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The possibility of changing two other features of the Official Method which add to its slowness might also be considered. It appears that official recognition should be given to the general practice of correcting Lintner values for the reducing power of the starch solution but not for that of the malt infusion. The correction for the latter is small and varies within narrow limits and for all practical purposes can be disregarded. It also seems desirable to return to the older method of carrying out the diastasis with 2 or 1 ml. of undiluted infusion and 100 ml. of starch solution in a 200 ml. flask. The dilution of the extract can be omitted with very slight loss of precision, and the use of smaller volumes of starch and the same size of flask for malts above 135° L. is advantageous in a routine laboratory. The adoption of these procedures effects a very considerable saving in the time required both for the actual determinations and the washing of glassware.

If any great improvement is to be made in the method for determining the diastatic powers of American malts, it appears that it will be necessary to make a radical change in the method of determining the maltose. Such a change was recently proposed by Gore and Steele (6), who describe a procedure involving the use of a modification of the Blish and Sandstedt ferricyanide method (2, pp. 218–221; 3 or 4). Their specifications, however, are not considered entirely satisfactory because the directions for making the malt infusion and the diastasis differ considerably from those of the Official Method; the neutralization of the digested starch solution and the use of large volumes for reduction and titration are unnecessary and time-consuming; and because the derivation of the conversion factor involves certain theoretical considerations of which no account is taken in the Official Method.

In the present paper a procedure is outlined for determining the maltose by means of the original semi-micro ferricyanide method of Blish and Sandstedt. There can be no doubt that this method is suitable for routine work since, as applied to the determination of the diastatic power of flour, it has received wide trial and is now recommended for adoption as an official method both by the American Association of Cereal Chemists, and by the Association of Official Agricultural Chemists. The method as applied to the determination of the Lintner values for malts has been in use for some time in three Canadian laboratories. The reduction in the time required to determine the reducing powers of the digested starch solutions is estimated as 50%, and there is general agreement that the method is more convenient and precise than the titration of boiling Fehling's solution.

In order to demonstrate the soundness of the ferricyanide method it is necessary to show: that under the conditions of the determination, the relation between the actual reducing power of digested starch solutions of various concentrations and their ferricyanide equivalents can be represented by a straight line having a slope of 45°; and that the ratio of the results obtained by the Official Method and the corresponding ferricyanide equivalents, for a series of malts covering a wide range of diastatic powers, is relatively constant. Data on these points are given in the experimental section.

There remains the problem of determining the factor for converting ferricyanide equivalents to degrees Lintner. Only a tentative solution can be offered. The difficulty lies in the fact that no one laboratory can state with assurance that its values, as obtained by the Official Method, are correct (*vide* Coleman (5)). The problem can best be solved by a co-operative investigation made in a number of laboratories, and it is hoped that the method outlined in this paper may prove sufficiently attractive to merit such an investigation by one or all of the three associations of chemists previously mentioned.

The Method

Malt Infusion and Starch Solution

Prepare the malt infusion and the buffered starch solution according to the Official Method of the American Society of Brewing Chemists (1, pp. 16–18).

Diastasis

Pipette 100 ml. of buffered starch solution into a 200 ml. volumetric flask and place it in a constant temperature bath maintained at 20° C. ($\pm 0.1°$). When the solution has reached a temperature of 20° C. add exactly 2 ml. of malt infusion with a precision grade pipette. Rotate the flask during the addition and immediately thereafter tilt the flask so that the solution runs up over the side of the neck against which the pipette was drained. Maintain the mixture at 20° C. Exactly 30 min. after starting to add the infusion add 10 ml. of 0.5 *N* sodium hydroxide solution from a fast flowing pipette. Mix, make up to the mark with distilled water and again mix thoroughly. If the malt has a diastatic power of over 135° L. make the diastasis by the same method but use 1 ml. instead of 2 ml. of infusion.

If the 2 ml. and 1 ml. pipettes are of precision grade and thoroughly clean, if the outside of the delivery tube is wiped with a clean cloth before the volume is adjusted, and if the pipette is drained against the wet side of the flask neck for exactly 15 sec. after free flow ceases, then no appreciable loss of precision results from the procedure outlined above.

Starch Blank

Prepare the starch blank by adding 10 ml. of 0.5 *N* sodium hydroxide solution to 100 ml. of buffered starch solution in a 200 ml. volumetric flask and make up to the mark with distilled water.

Determination of Reducing Power

Determine the reducing power of the digested starch solution and of the starch blank by means of the ferricyanide method of Blish and Sandstedt (2 pp. 218–221; 3 or 4), using 5 ml. of solution and 10 ml. of ferricyanide reagent.

The reductions may be made in 100 ml. wide-mouth hard glass Erlenmeyer flasks covered with small crucibles. Results obtained with this technique and with the original one agree to within 0.01 ml. The modification saves time by eliminating the transfer of the reduction mixture from test tube to flask and permits the determinations to be run in batches of four or

six, the first batch being titrated while the second is in the boiling water bath, and so on. This leads to a more continuous procedure and greater accuracy (Compare Putman, Blish and Sandstedt (9)).

Calculation

Subtract the number of millilitres of sodium thiosulphate required to titrate the digested starch reduction mixture from the number of millilitres required to titrate the starch blank reduction mixture. The resulting number is the ferricyanide equivalent of the diastatic power of the malt. To convert the results to degrees Lintner multiply the ferricyanide equivalent by 18 when 2 ml. of malt infusion was used and by 36 when 1 ml. was used.

For routine laboratories it may be more convenient to use 0.0278 *N* sodium thiosulphate solution for which the conversion factors are 10 and 20.

Standardization of Sodium Thiosulphate Solution

As Blish and Sandstedt (3) have pointed out, standardization of the sodium thiosulphate solution is unnecessary if it is made up according to their directions. However, if it is desirable to standardize it for special work, the use of potassium iodate, recommended by Hanes (7), is rapid and trustworthy.

Experimental

Relation Between Reducing Powers of Digested Starch Solutions and Corresponding Ferricyanide Equivalents

An infusion was prepared from a malt having a diastatic power of approximately 160° L., and after diluting 1 to 5, aliquots of 10 ml. were used to digest 100 ml. quantities of buffered starch solution. After adding 10 ml. of 0.5 *N* sodium hydroxide all the solutions were mixed without further dilution. A supply of starch-infusion blank solution was prepared in a similar manner. Various quantities of the digested starch solution were measured into tared 200 ml. volumetric flasks, weighed and then made up to 120 ml. with the starch-infusion blank solution. Finally, the flasks were made to the mark with distilled water and the reducing powers of the solutions were determined by the ferricyanide method.

TABLE I

RELATION BETWEEN REDUCING SUBSTANCES PRESENT AND FOUND IN DIGESTED STARCH SOLUTIONS

Weight of digested starch solution, gm.	Ferricyanide equivalent, ml.	Reducing power, °L.		Difference, °L.
		Present	Found	
104.45	7.59	136.6	136.6	0.0
97.38	7.14	127.4	128.5	−1.1
90.35	6.55	118.1	117.9	0.2
83.28	6.08	108.9	109.4	−0.5
76.30	5.52	99.7	99.4	0.3
69.31	5.06	90.5	91.1	−0.6
62.27	4.50	81.4	81.0	0.4
55.22	4.01	72.2	72.2	0.0
48.17	3.46	62.8	62.3	0.5
42.14	3.06	55.1	55.1	0.0

The relative quantities of reducing substances present in each solution, calculated from the weights of digested starch solution, and the ferricyanide equivalents found are reported in Table I. The data have been converted to degrees Lintner to show the range of Lintner values which the experiment covers. Fig. 1 shows the graph obtained by plotting calculated values against values found.

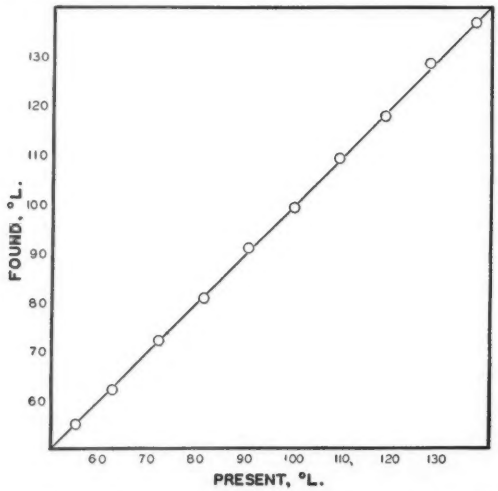

FIG. 1. *The relation between reducing substances present and found in digested starch solutions.*

It will be obvious that the experimental results are applicable to a double range of Lintner values, namely, from 55° to 136°, and from 110° to 272°, because the amounts of reducing substances produced during diastasis fall in the same range irrespective of whether 2 ml. or 1 ml. of infusion is used. The data show that under the conditions of the determinations of the diastatic powers of malts, the ferricyanide method provides an accurate measure of the reducing powers of the digested starch solutions.

Relation Between Results Obtained with Official and Ferricyanide Methods

The results obtained by following the directions for the titration, given in the Official Method, were not sufficiently precise, and it was therefore necessary to superimpose on them the more precise specifications of Lane and Eynon (8). The possible bias introduced by this change was overcome by standardizing the Fehling's solution by titrating it under exactly the same conditions with a solution containing 2 mg. of invert sugar per millilitre, and by adjusting the concentration of the Fehling's solution to correspond with a titre of 25.65 ml., the value given in Lane and Eynon's table for invert sugar (8). With the exception of this modification the directions given in the Official Method were followed exactly.

Eight malts were selected with diastatic powers between 70° and 140° L. Infusions were prepared from duplicate samples of each malt, and duplicate diastases and starch-infusion blanks were made using 2 ml. of each infusion. The reducing power of each digested starch solution and the corresponding blank correction were determined by both the Official and ferricyanide methods. A similar study was also made with eight malts with diastatic powers above 135° L. With these malts it seemed unwise to use the same digested starch solutions for both methods because the Official Method specifies that the diastasis shall be made in a 250 ml. flask with 10 ml. of diluted infusion, 200 ml. of buffered starch solution and 20 ml. of 0.5 *N* sodium hydroxide solution, whereas the ferricyanide method requires the use of a 200 ml. flask, 1 ml. of infusion, 100 ml. of buffered starch solution, and 10 ml. of 0.5 *N* sodium hydroxide solution. The former procedure is unsatisfactory for the latter method because it increases the concentration of reducing substances and thus lowers the upper limit which can be obtained with the ferricyanide reagent, and because it involves a change in the alkalinity of the solution which affects the relation between the ferricyanide equivalent and the actual reducing power of the solution. For these reasons separate diastases were made for each method in the second study.

The results of the two studies are reported in Table II. The data represent the means of determinations made on quadruplicate digested starch solutions.

TABLE II

COMPARISON OF RESULTS OBTAINED BY THE OFFICIAL AND FERRICYANIDE METHODS

Malt No.	Official method, °L.	Ferricyanide equivalent, ml. 0.05 *N*	Ratio, °L. Official to ferricyanide equivalent	Ferricyanide method, °L.*	Difference, °L.
1	72.4	3.88	18.66	69.8	2.6
2	79.7	4.34	18.36	78.2	1.5
3	88.7	4.85	18.29	87.3	1.4
4	98.9	5.42	18.25	97.5	1.4
5	104.3	5.73	18.20	103.1	1.2
6	118.0	6.60	17.88	118.8	−0.8
7	129.8	7.38	17.59	132.8	−3.0
8	137.4	7.72	17.79	139.0	−1.6
Mean	103.7	5.74	18.07	103.3	0.4
9	157.6	4.32	36.48	155.5	2.1
10	162.5	4.43	36.68	159.5	3.0
11	169.8	4.62	36.75	166.3	3.5
12	173.3	4.69	36.95	168.8	4.5
13	173.6	4.76	36.47	171.3	2.3
14	176.6	4.85	36.41	174.6	2.0
15	179.5	4.89	36.71	175.9	2.2
16	185.3	4.99	37.13	179.5	5.8
Mean	172.3	4.69	36.74	169.0	3.3

* *Ferricyanide equivalents were converted to degrees Lintner by multiplying by factors of 18 and 36 in the upper and lower halves of the table, respectively.*

Factors of 18 and 36 were selected for converting ferricyanide equivalents to degrees Lintner since the differences between these whole numbers and the ratios corresponding to 100° and 200° L. appeared to be insignificant.

It will be observed that the data in the upper half of the table show that the ratios between the results of the Official Method and the corresponding ferricyanide equivalents decrease fairly steadily with increasing diastatic power. This point is not so well illustrated by the second study in which the experimental errors were greater and the malts covered a narrower range of diastatic powers. It is worth noting, however, that in the second study the mean factor of 36.74 for a diastatic power of 172° L., which corresponds to a factor of 18.37 for a diastatic power of 86° L. in the lower range, agrees fairly well with the data reported in the upper half of the table.

In view of the data on the accuracy of the ferricyanide method reported in Table I, and for reasons given below, it seems fair to attribute the discrepancies between results obtained by the two methods almost wholly to the inaccuracy of the Official Method. When the ferricyanide equivalents are converted to degrees Lintner using a constant factor it turns out that, within each range, the Official Method overestimates the lower diastatic powers and underestimates the upper ones.

The quantity of maltose required to reduce a constant volume of Fehling's solution falls with increasing final volume of the reaction mixture, *i.e.*, with decreasing concentration of the unknown solution (Lane and Eynon (8)). When the titration of Fehling's solution was first adopted for the determination of Lintner values no correction was applied for this volume effect presumably because it was small. However, when the titration is used under the conditions outlined by the Official Method, this error becomes appreciable owing to the greater variation in the final volume of the reaction mixture caused by the method of determining the blank correction. In these circumstances it will be obvious that if a standard value is taken in the middle of the Lintner scale for each range, then the Official Method will overestimate the lower values and underestimate the upper ones, and as a net result the curvilinear nature of the relation expressed by Kjeldahl's law will be overemphasized.

The Precision of the Proposed Method

The proposed method has been tested under a routine schedule, which required that the malts for several days' analyses be split into duplicate samples and arranged in random order. The analyst then made 12 single determinations, in random order, per day and washed all glassware used. Under these conditions the differences between duplicate samples are the result of all errors inherent in the method. In the National Research Laboratories, Ottawa, the standard error of the mean of duplicate determinations was found to be $\pm 0.94°$ L. for 30 pairs of determinations involving the use of 2 ml. of infusion, and $\pm 1.06°$ L. for 84 pairs involving the use of 1 ml. of infusion. In the malting laboratory at the University of Manitoba, Winnipeg,

under the same conditions, the standard error for 112 pairs of determinations was found to be $\pm 1.07°$ L. These data appear to represent a satisfactory level of precision for routine analysis.

References

1. American Society of Brewing Chemists. Official Methods. 1936.
2. Association of Official Agricultural Chemists. Methods of Analysis. 1935.
3. Blish, M. J. and Sandstedt, R. M. Cereal Chem. 10 : 189-202. 1933.
4. Blish, M. J. J. Assoc. Official Agr. Chem. 16 : 497-504. 1933.
5. Coleman, D. A. Cereal Chem. 13 : 669-674. 1936.
6. Gore, H. C. and Steele, H. K. Ind. Eng. Chem., Anal. Ed. 7 : 324-326. 1935.
7. Hanes, C. S. Biochem. J. 23 : 99-106. 1929.
8. Lane, J. H. and Eynon, L. Soc. Chem. Ind. 42 : 32T-37T. 1923.
9. Putnam, H. W., Blish, M. J. and Sandstedt, R. M. Cereal Chem. 12 : 494-504. 1935.

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BIOLOGICAL NOTES ON THE CHRYSOPIDAE[1]

BY WM. L. PUTMAN[2]

Abstract

This paper presents further biological and ecological data on the Chrysopidae gathered during a study of the predators of the oriental fruit moth. The species concerned are *Chrysopa rufilabris* Burm.; *C. plorabunda* Fitch; *C. downesi* R. C. Smith; *C. oculata* Say; *C. nigricornis* Burm.; *Meleoma signoretti* Fitch and *M. emuncta* Fitch. Data on life history include relation of development to temperature, number of generations, method of overwintering, and oviposition. The early stages of *C. downesi*, *M. signoretti* and *M. emuncta* are described. Seasonal prevalence and fluctuations in abundance from year to year are discussed and notes are given on natural control agencies. The value of chrysopids in the biological control of the fruit moth is summarized, with the conclusion that only in exceptional seasons are they of appreciable importance.

In a previous paper (1) the writer gave some account of the value of chrysopids as predators of the oriental fruit moth (*Grapholitha molesta* Busck), together with some notes on their habits and life histories. This paper presents further details of studies in which most attention was given to *Chrysopa rufilabris* Burm., *C. plorabunda* Fitch, and *Meleoma signoretti* Fitch, these being the most abundant and the only species of appreciable importance as predators of the fruit moth. More or less incidental notes were also made on *C. oculata* Say, *C. downesi* Smith, *C. nigricornis* Burm., and *M. emuncta* (Fitch). There is some doubt concerning the identity of the species here called *Chrysopa downesi* which is discussed on a later page.

Life History

All the life-history studies were carried on in the screened insectaries at St. Davids and Vineland Station.

Incubation period. It was possible to determine the incubation period of only a small number of eggs as very few were laid in captivity. The length of the period, with the mean daily temperature determined from thermograph records is given in Table I.

TABLE I
INCUBATION PERIOD OF CHRYSOPIDS

Species	No. of eggs	Length of period, days	Mean daily temperature, °F.
C. rufilabris	26	6 – 7	68.6 – 64.8
C. plorabunda	12	5 – 6	73.3 – 66.6
M. signoretti	5	9	66.1

Larval period. The larvae were reared in glass shell vials plugged with cotton. They were supplied daily with fresh eggs or larvae of the fruit moth

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or Mediterranean flour moth (*Ephestia kuehniella* Zell.). The duration of the three larval instars was recorded for a few larvae and appeared to be approximately equal. The length of the complete period in relation to the mean daily temperature is given in Table II.

TABLE II

LARVAL PERIOD OF CHRYSOPIDS

Mean daily temperature, °F.	No. of larvae	Larval period, days	
		Extremes	Average
Chrysopa rufilabris Burm.			
77 – 77.9	3	10	10.0
76 – 76.9	4	9 – 13	10.8
75 – 75.9	26	9 – 12	10.0
74 – 74.9	52	10 – 14	11.5
73 – 73.9	43	9 – 14	11.1
72 – 72.9	35	10 – 15	11.9
71 – 71.9	49	11 – 15	12.6
70 – 70.9	45	11 – 15	12.7
69 – 69.9	18	11 – 15	13.0
68 – 68.9	15	12 – 15	13.3
67 – 67.9	8	12 – 14	12.8
66 – 66.9	1	14	14.0
65 – 65.9	9	14 – 18	15.8
63 – 63.9	3	16 – 18	16.7
62 – 62.9	1	17	17.0
59 – 59.9	2	24	24.0
Chrysopa plorabunda Fitch			
77 – 77.9	1	8	8.0
76 – 76.9	1	9	9.0
74 – 74.9	8	10 – 12	10.5
73 – 73.9	2	10	10.0
71 – 71.9	4	10 – 15	12.5
70 – 70.9	11	10 – 13	11.5
69 – 69.9	10	12 – 13	12.4
68 – 68.9	10	11 – 14	13.2
67 – 67.9	2	12 – 15	13.5
66 – 66.9	1	15	15.0
65 – 65.9	5	16	16.0
64 – 64.9	5	16 – 18	17.0
63 – 63.9	2	17	17.0
61 – 61.9	4	18 – 20	19.5
60 – 60.9	4	19	19.0
59 – 59.9	1	23	23.0
Meleoma signoretti Fitch			
73 – 73.9	1	15	15.0
71 – 71.9	4	15 – 19	16.7
70 – 70.9	3	15 – 18	16.0
69 – 69.9	8	17 – 18	17.6
68 – 68.9	15	17 – 19	18.5
67 – 67.9	2	18 – 19	18.5

Pupal period. The cocoons were left in the vials in which they were spun. There was considerable mortality in the cocoons, ranging from 13 to 37% based on 41 and 187 individuals respectively. Moisture was supplied by wetting the plugs of the vials occasionally. The period as given in Table III includes the prepupal stage, pupation occurring from three to six or more days after the cocoon was spun.

TABLE III

PUPAL PERIOD OF CHRYSOPIDS

Mean daily temperature, °F.	No. of pupae	Pupal period, days	
		Extremes	Average
Chrysopa rufilabris Burm.			
77 – 77.9	3	10 – 11	10.3
76 – 76.9	7	9 – 13	10.9
75 – 75.9	5	10 – 12	11.5
74 – 74.9	13	11 – 13	11.4
73 – 73.9	29	11 – 14	11.7
72 – 72.9	20	11 – 16	13.0
71 – 71.9	27	11 – 15	14.0
70 – 70.9	21	13 – 16	14.4
69 – 69.9	1	15	15.0
68 – 68.9	10	13 – 17	15.6
67 – 67.9	12	16 – 18	16.6
66 – 66.9	2	16 – 17	16.5
65 – 65.9	8	17 – 20	19.0
64 – 64.9	9	18 – 21	19.3
74 – 74.9	2	10 – 11	10.5
73 – 73.9	1	10	10.0
71 – 71.9	1	11	11.0
70 – 70.9	6	11 – 14	12.2
69 – 69.9	2	13	13.0
68 – 68.9	2	13 – 14	13.5
67 – 67.9	4	12 – 14	13.3
65 – 65.9	18	14 – 19	18.0
64 – 64.9	1	19	19.0
61 – 61.9	2	20 – 21	20.5
60 – 60.9	1	19	19.0

Oviposition and longevity of adults. It was very difficult to get adults of *C. rufilabris* emerging in the insectary to oviposit, although cages of various types were used and the insects supplied with water and aphids, sugar solution, and yeast. A few eggs were obtained from cages placed on aphid-infested trees but the number normally laid is not known. When supplied with aphids, adults of this species lived as long as 45 days, but they died quickly when given water only.

C. plorabunda was much more easily handled in captivity and usually oviposited when caged on aphid-infested twigs. Adults of the summer generations deposited from 18 to 114 eggs and lived as long as 42 days. Overwintering adults laid more than 56 eggs over a period of five weeks. An examination of the undeveloped ovaries of this species showed a potential production of at least 144 eggs.

Seasonal life history. *C. rufilabris* overwintered in the prepupal stage within the cocoons. These are spun among the leaves, with which they fall, on the twigs, and in considerable numbers inside the curls of the thin outer bark. The exact dates of spring emergence were not determined but they appeared to be early in June, as eggs were found as early as June 10. There appeared to be two complete generations and probably a partial third, but these were not distinguishable in the field because of the long oviposition period and the great variation in the length of the larval stages under conditions of food scarcity. The number of eggs present in the peach orchards usually increased rapidly from about the middle of June and reached a maximum about the middle of July, after which oviposition decreased rapidly. Eggs were very scarce after early August, although a few could be found till nearly the middle of September. All the eggs found up to and including the time of maximum deposition were laid by the spring brood of adults, and while in some years, particularly in 1933, there was some indication of a slight increase in the egg population in early August, the number laid by the first brood adults was very small and much less than that of the spring brood. The presence of the first brood in considerable numbers was shown by the bait pail catches, so it appeared that the decline in egg population was due to the reduced fecundity of the females of this brood. In the writer's previous paper it was conjectured that lack of food for the larvae was responsible, but an equally probable cause was insufficient nourishment of the adults. The experiments in connection with oviposition outlined above appear to show that aphids are necessary for the proper development of the ovaries. Green peach aphids, *Myzus persicae* Sulz., were usually common in the orchards during the greater part of June and a few usually remained until July, while the spring brood of *rufilabris* was active, but all migrated to other hosts before the appearance of first brood adults.

C. plorabunda hibernated in the imaginal state. Several were successfully wintered on the earth floor of the insectary under an inverted flower-pot. While none were actually discovered in midwinter, they were found in late

fall and early spring among dead grass and fallen leaves, in strawberry beds and in outbuildings. On warm days they were often active until late November and were seen in flight as early as March 22. On the approach of cold weather, in late October and November, the usual green color of the species changed to yellow mottled with brown, but reappeared early in May after they emerged from hibernation and began to feed. Both sexes hibernated, the females with undeveloped ovaries, and fertilization took place in the spring. In cages, oviposition commenced about the first of June and continued nearly to July. In the orchards, eggs were not found before June 27 and in some years not until nearly a month later. Apparently the adults did not hibernate in the orchards because of lack of shelter, and did not invade them in noticeable numbers until the time of the late spring brood or early first brood oviposition. After this time eggs could be found during most of the summer, sometimes till September 11, but there were no consistent periods of maximum oviposition and the broods could not be distinguished. As this species produces eggs earlier in the season and the developmental period is somewhat shorter, three complete generations are possible, but it is doubtful whether the third is very large.

Meleoma signoretti passed the winter as a prepupa within the cocoon. Adults emerged between June 2 and 5 from a few cocoons overwintered in the insectary. In 1932, the only year in which any appreciable numbers were present, the first eggs were found in the orchards on July 5, the maximum number on July 20 and the last on August 24. There was one complete generation and, at least in some years, a partial second. In 1931, first brood adults were taken in the bait pails from August 18 to September 8, while in 1932, when the species was much more abundant, none were taken, and larvae maturing as early as August 7 did not emerge as adults that season.

Food Habits of the Larvae

Larvae of *C. rufilabris* were seen in the orchards attacking eggs and larvae of the oriental fruit moth, nymphs of the cottony peach scale (*Pulvinaria amygdali* Ckll.), European fruit lecanium (*Eulecanium corni* Bouché), white fly (*Trialeurodes vaporariorum* Westw.), the leaf-hopper, *Erythroneura obliqua* Say, and all stages of the European red mite (*Paratetranychus pilosus* C. & F.). They would apparently destroy almost any soft-bodied insect not too large or active to handle, but could not be reared satisfactorily on green apple aphids (*Aphis pomi* DeGeer) and also refused the woolly species of aphids.

There was less opportunity for observing the food preferences of *C. plorabunda* and *M. signoretti*, but these species and *C. downesi* ate fruit-moth and flour-moth eggs and larvae and aphids in the insectary and were apparently similar to *rufilabris* in their choice. *C. oculata* appeared to be chiefly an aphid feeder, as only a few larvae could be induced to accept fruit-moth eggs and then only when starved. The habits of *C. nigricornis* appeared to be very similar.

Fluctuations in Abundance

As part of an ecological study of the oriental fruit moth, population studies of chrysopid eggs were made from 1931 to 1935 at four selected orchards in the Niagara Peninsula. These were located at St. Davids, Niagara-on-the-Lake, Vineland Station, and Grimsby Beach, covering about 22 miles of the fruit belt. Briefly, the procedure was to make one-hour counts at chosen points in the mature orchard at weekly intervals from approximately June 1 to September 10. Duplicate counts were also made on young trees at each point, but because of the variation in the age of the trees and the necessity of choosing a new block as the original one became too old, it was thought that the figures were not comparable and were discarded. Approximately a thousand leaves were carefully examined at each hourly count and all fruit-moth eggs and other insects also recorded. The chrysopid eggs were collected and reared to determine the species. In Table IV the combined counts for each season are given.

TABLE IV

CHRYSOPID EGG POPULATION IN PEACH ORCHARDS

	1931	1932	1933	1934	1935
C. rufilabris	508	11	83	262	119
C. plorabunda	90	19	5	16	19
C. nigricornis	—	32*	—	—	—
C. oculata	—	6	18	68	19
M. signoretti	—	34	3	—	1

** 1 cluster of 32 eggs.*

Unfortunately no counts were made in 1930 when chrysopids were more abundant than in any succeeding year. A perusal of the table will show a great variation in both the abundance of each species during the five-year period and the relative numbers of the species in each year. *C. rufilabris* was by far the most prevalent species in every year except 1932, when it was relatively scarce; it occurred in greatest abundance in 1931 and was again very common in 1934. *C. plorabunda* was always much less abundant except during 1932, and was also most prevalent in 1931. *M. signoretti*, usually an uncommon species, suddenly became quite common in 1932, but very few were found during succeeding years. The few eggs of *C. oculata* were collected largely during June, while aphids were still present on the trees, and it should be noted that the greatest numbers were found in 1934, a year of unusually heavy aphid infestation. Eggs of *C. nigricornis* were found on only one occasion.

The larvae of *rufilabris*, *plorabunda* and *signoretti* were commonly observed on the trees in numbers roughly proportionate to the egg populations. Exceedingly few *oculata* were found, the larvae apparently migrating to the soil or low vegetation. One or two larvae of *C. downesi* were collected and a few eggs found in another orchard.

Natural Control Factors

Infertility of eggs. Approximately 5 to 9% of the eggs collected in the orchards failed to hatch. These figures were derived from collections of 103 and 180 eggs respectively. Some of this mortality might have been due to injury in collecting, but many eggs appeared to be infertile.

Egg parasitism. Parasitism of the eggs by *Trichogramma minutum* Riley ranged from traces in 1933 and 1934 to 5% of counts of 290 and 179 eggs in 1932 and 1935 respectively, and 7% of 987 eggs in 1931. In individual orchards it reached 12% of 221 eggs examined in 1931 and 50 examined in 1932. Chrysopid eggs in years of abundance may be of some importance as a reservoir in which a stock of *Trichogramma* is built up to attack the later generations of the fruit moth.

Food supply of larvae. Lack of sufficient food appeared to be one of the most important factors limiting the chrysopid population. In some years chrysopid eggs were often the most prevalent form of life on the trees and under such conditions very few larvae could survive.

Pupal parasites. These are not properly pupal parasites as they attack the prepupa. The only primary parasite discovered was *Chrysopoctonus rileyi* Ashm. which was reared several times from summer generation cocoons of *rufilabris*, *plorabunda*, and *nigricornis* and probably attacks all species. The most abundant species was *Hemiteles tenellus* Say, reared many times from cocoons of both summer and overwintering generations of *rufilabris* and *plorabunda*. An examination of the contents of the cocoons proved that this species was hyperparasitic, probably on *Chrysopoctonus*, and in view of its abundance must be a decided check on parasitism by the latter species. One specimen of another hyperparasite, *Perilampus chrysopae* Cwfd., was obtained, and a few undetermined chalcids emerged from another cocoon. It was impossible to determine the extent of parasitism at any one time because of the scarcity of cocoons, but a lot of 31 old cocoons collected in 1932 from peach trunks, and representing all generations for two years or more showed a parasitism of approximately 50%.

Destruction of cocoons by predators. Some of the cocoons found on the trunks had been torn open by some predator and the pupae devoured. Some coleopterous larvae resembling *Tenebroides* were found in the same location and may have been responsible.

Natural mortality within the cocoon. Many cocoons were found containing dead pupae or prepupae. Among those collected on the trunks this natural mortality reached approximately 26%.

Food supply of adults. The possibility that a deficient food supply may limit the fecundity of the females has been discussed on a previous page.

Summary. The most important factors limiting the chrysopid population are lack of food for the larvae and possibly for the adults, and parasitism; but the manner in which these and probably other unknown influences, such as weather conditions, cause the great fluctuations in numbers is not known.

Descriptions of Immature Stages

The early stages of most of the species concerned have been well described and figured by Smith (2) and only those not previously known are described here.

Chrysopa downesi SMITH

There may be some doubt concerning the identity of the species here called *downesi*. A small series was identified as this species by its author, Dr. R. C. Smith, but none of the hundred or more examined by the present writer have the black band over the genae mentioned in the original description (3), but have a red band only, in this respect agreeing with *harrisii* Fitch. There is some variation in size and the relative widths of the wings but only one species appears to be represented. The adults in life have a characteristic bright, deep green color which soon disappears in mounted specimens.

Egg. Stalked; yellowish-green; indistinguishable from that of *plorabunda*.

Larva. Unfortunately no detailed description of this species was made, but the only noticeable distinctions between it and that of *plorabunda* were the markings of the head. These consisted of two longitudinal bands converging somewhat toward the base of the head, as in *plorabunda*, but of uniform width throughout and not expanding at the base as in the latter species.

Cocoon. Oblong-spherical, of pale yellow-green silk. Cocoons of other species may appear greenish but this is due to the enclosed pupa. Length 3.2 mm.; width 2.8 mm.

Meleoma signoretti FITCH

Egg. Of typical chrysopid type; stalked; deposited singly.

First instar larva. General color pale. Head with two conspicuous longitudinal, somewhat reniform black marks, concave toward the middle line, very similar to the second instar. Smith's figure ((2), Plate LXXIV) doubtfully referred to this species, is apparently correct. Thoracic segments each with two large dark spots. Abdomen with obscure darker markings. Tubercles with long, curved pale setae. Legs pale, apices of segments ringed with dusky. Width of head, 0.39 mm.; length of mandibles, 0.39 mm.; antennae, 0.57 mm.; longest setae, 0.52 mm.; total length, 2.07 mm.

Second instar larva. (Fig. 1, a). Head similar to first instar but spots more angular; jaws pale amber, darker apically; antennae and palpi pale brown. Thorax pale yellowish; prothorax with two dark longitudinal im-

FIG. 1. *a. Meleoma signoretti; second instar larva. b. M. signoretti; third instar larva. c. M. emuncta; third instar larva.*

pressions (sclerites) and marked with pale reddish; meso- and metathorax each with two brown marks on either side, the posterior larger and extending diagonally outward and backward to small black impressions; pale reddish spots external to brown ones; dorsal vessel brown with lighter brown irregular stripes on either side; tubercles pale marked with reddish. First abdominal segment without tubercles; those of second segment marked with brown. Abdomen pale yellow, with irregular broken brown markings tending to form two bands laterally which become darker and converge toward the caudal extremity; brown markings interspersed with pale reddish. Thoracic and abdominal tubercles well developed, with curved pale setae. Legs pale. Width of head, 0.74 mm.; length of mandibles, 0.59 mm.; antennae, 0.98 mm.; longest setae, 0.50 mm.; total length, about 5 mm.

Third instar larva. (Fig. 1, b.) Head with two pairs of curved black marks, the inner on the anterior part of the head, extending forward, diverging and angled outward to the base of the jaws; outer pair shorter and broader, not extending as far anteriorly and merging into broad pale brown bands extending posteriorly to the base of the head; from the outer side of these, similar broad bands extend diagonally outward and forward to the eyes; a dark band extending along side of head from base nearly to eyes; eyes black. Jaws amber brown, darker apically. Antennae and palpi pale brown. Thorax and abdomen similar to second instar but markings paler and more diffuse; black impressions on prothorax divided longitudinally and a central black spot above dorsal vessel. Width of head, 1.04 mm.; length of mandibles, 0.95 mm.; antennae, 1.45 mm.; longest setae, 0.75 mm.; total length, approximately 10 mm.

Cocoon. Somewhat oblong spherical; white; loose outer layer sometimes with a reddish tint when new; length 3.8 mm.; width 3.3 mm.

Meleoma emuncta (FITCH)

Egg. Indistinguishable from that of *M. signoretti;* laid singly.

First instar larva. Head with two large longitudinal black marks which meet along the middle line, leaving triangular light areas at base and apex of head; a narrow black line in the light areas on either side of the large marks extending from the base half the length of the head; regions about the eyes black; mandibles amber-colored. Thorax and abdomen pale reddish brown; lateral tubercles white except second abdominal which are dark; setae long and curved, pale. Legs pale.

Second instar larva. Head similar to first instar; ground color pale yellowish gray. Thorax pale yellowish; prothorax marked with pale reddish and with two longitudinal black impressions; mesothorax and metathorax marked with brown, tending to form two oblique bands converging posteriorly on each segment with a smaller spot external to them; sides of mesothorax below and behind lateral tubercles dark; tubercles pale, marked with pale reddish, large and prominent. Abdomen darker yellowish, finely marked with brown, more heavily and tending to form two bands toward the caudal extremity;

dorsal vessel pale grayish brown, bounded by paler lateral areas; sides of second abdominal segment, including lateral tubercles, dark brown to black; tubercles on other segments finely spotted with brown on bases; setae on all tubercles long, curved, partly black.

Third instar larva. (Fig. 1, c.) Head pale yellowish marked with dark brown or black; from centre of head two black bars extend parallel anteriorly and then diverge sharply toward bases of jaws; a median triangular black spot between the bands near the anterior margin; two short oblique bars external to central bands; from these bars paler brown bands extend forward to bases of antennae and backward to base of head where they become much broader and give off similar bands obliquely outward nearly to eyes, enlarged, darker, and frequently somewhat crescent-shaped at the apices. Mandibles amber-colored, darker apically. Antennae and palpi pale brown. Thorax and abdomen similar to second instar, but brown markings paler and interspersed with pale reddish; sides of mesothorax pale. Legs pale. Width of head, 1.14 mm.; length of mandibles, 1.00 mm.; antennae, 1.54 mm.; total length, about 10 mm.

Cocoon. Similar to that of *M. signoretti.* Length, 4.00 mm.; width, 3.3 mm.

The larvae of *Meleoma* are easily distinguished from those of *Chrysopa* by their yellow-brown color and by their head markings. *Emuncta* larvae can be separated from *signoretti* by the median triangular spot near the apex of the head in the former species.

Influence of Chrysopids on the Oriental Fruit Moth

The only species with such habits and occurring in such numbers that they are potential factors of importance in the control of the fruit moth are *Chrysopa rufilabris*, *C. plorabunda*, and *Meleoma signoretti*. There is little to add to the writer's account of their destruction of eggs given in his previous paper (1), but they have since been found occasionally attacking larvae in the field. Unfortunately we still have no method of giving a numerical value to their influence, but there is no doubt that in 1931, and still more so in 1930, chrysopids were of considerable importance in the control of the moth, but from 1932 to 1935 they were apparently of minor value. The period of greatest abundance coincides very closely with the time the second brood eggs are laid and the greatest check is exerted on this brood.

References

1. PUTMAN, W. L. Chrysopids as a factor in the natural control of the oriental fruit moth. Can. Ent. 63 : 121-126. 1932.
2. SMITH, ROGER C. The biology of the Chrysopidae. Cornell Agr. Expt. Sta. Mem. 58 : 1291-1372. 1922.
3. SMITH, ROGER C. The Chrysopidae of Canada. Ann. Entomol. Soc. Am. 25 : 579-600. 1932.

STUDIES ON THE HETEROPHYID TREMATODE *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART II. LIFE HISTORY AND BIONOMICS[1]

By Thomas W. M. Cameron[2]

Abstract

The eggs of the trematode pass into water, embryonate and are swallowed by the snail, *Goniobasis livescens*. They do not hatch in water. In the snail, multiplication through redia and daughter redia stages takes place and cercariae, with long flanged tails and pigmented eyespots, are produced. These escape into the water, but have a free life of less than 48 hours. To survive during this period, they must penetrate the skin of a fish, a great variety of which are successful intermediaries. In the muscle of the fish, the cercaria encysts to become metacercaria, which is however not immediately infective. Infection of the definitive host is by ingestion of the uncooked fish.

When *Apophallus venustus* was found to be endemic in the lower Ottawa Valley, preparations were made to attempt to elucidate the life cycle. While the life history of all the species in this genus was quite unknown, it was known that, in such other members of the family as had been studied, two successive intermediaries were essential. The second of these was always a fish, and the first an operculate snail.

Accordingly, a large number of freshwater fish was obtained locally and fed to kittens which had been raised in the college and had never been fed on fish. With a very few exceptions, every species obtained was found to be infective. *Apophallus venustus* was obviously, like the other members of the family, carried by fish.

The search for the snail intermediary was more difficult. By analogy from what was known of the other members of this family, it should prove to be an operculate snail. Moreover, all the cercariae already known from the Heterophyidae have pigmented eye-spots and peculiar flanges on their tails. Finally, the high incidence of infection in the fish suggested that the snail vector would be common locally and that a large percentage would be infected.

Search was accordingly made in the first instance for common species of operculate snails in the Ottawa River. Five species were found and I wish to acknowledge the invaluable assistance of Mr. A. LaRocque, of the National Museum, Ottawa, in identifying these.

The commonest local operculate snail was *Amnicola limosporata* which was found in thousands on the stems of various water weeds. Several hundreds of these snails were crushed and examined microscopically. No trace of any kind of trematode larva was found.

[1] *Manuscript received November 3, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

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Campeloma decisum, a large species living on decaying vegetable matter, was also collected in some numbers, although it was much less common. No cercariae were found in this snail either.

Bulimus (Bithynia) tentaculatus is also very common locally, although it is a species introduced from Europe. This made it an improbable intermediate host for an endemic American trematode; nevertheless large numbers were examined but all proved negative.

These three species were subjected to artificial infection without result.

A species of *Pleurocerca*, a St. Lawrence form occurring in the Ottawa River only at its point of junction with the St. Lawrence River at Lake St. Louis, was obtained in small numbers. None was infected.

The remaining species, *Goniobasis livescens*, was first collected from stones just off Lynch Island, two miles east of Ste. Anne de Bellevue. It was present in large numbers and an examination of the first batch of 82 snails showed that 31% were infected with a cercaria which was very similar to that described by other workers from various species of Heterophyid trematodes. It seemed probable that this was the larval stage of *Apophallus venustus*. No other species of Heterophyid has been found locally in wild or domestic mammals or birds, and no other heterophyid cercaria has been found in any other species of local snail, although large numbers of all the common species, both operculate and non-operculate, have been carefully examined by my colleagues and myself in the laboratory during the past three years in connection with this and other trematode life cycles.

Positive proof of the life history of a trematode depends upon the production of all stages in the laboratory, in animals raised under circumstances which absolutely preclude any accidental contamination. In this particular case, it postulates laboratory-bred cats, laboratory-bred fish and laboratory-bred snails. The first of these conditions was easily fulfilled and the cats used have definitely not been exposed to natural infection. Attempts to breed suitable fish (such as catfish and black bass) in the laboratory under conditions which would satisfy these postulates, were unsuccessful and this was likewise the case with *Goniobasis livescens*.

Early in 1935, a large number of very young catfish had been collected and kept in the aquarium at the Institute of Parasitology. A number of these died from one cause or another but no encysted cercaria could be found on compressing the whole fish in a trichina compressorium. Others were later subjected to infection with the cercariae emerging from the snails and the larval flukes observed actively penetrating the skin of the fish. Experimentally infected fish were killed and examined from time to time and the encysted cercariae in the flesh were observed to develop and require some time to become mature. Negative results followed the feeding of cats with catfish containing young cysts and it seems reasonably sure that no previous infection with this fluke was present in the fish. One of these fish, a month

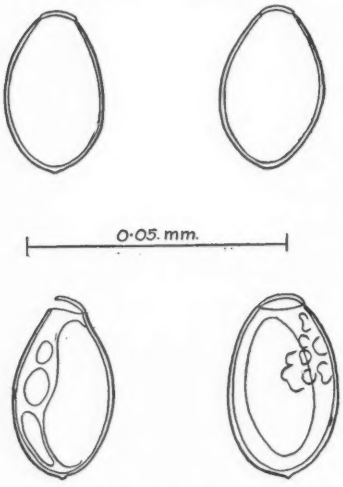
after its last exposure to infection, was fed to a kitten; three weeks later the cat was autopsied and *Apophallus venustus* found in its intestine. These experiments did not, however, furnish absolutely conclusive proof of identity.

A number of catfish was collected from an artificial pond on the grounds of Mr. J. J. Harpell, of the Garden City Press, Gardenvale, Quebec. These were young fish, bred in this pond which contained no operculate snails of any kind. They were retained in the laboratory for a month after experimental infection, and then fed to a cat. The result was positive. At the same time, a number of catfish from the same pond was collected and fed directly to another cat, without being exposed to the cercariae; the control was negative.

Thus the entire life cycle has not been observed in an entire experimental series. However, the high percentage of infected *Goniobasis livescens*, the presence of a single species of heterophyid cercaria in the area, and of a single species of adult heterophyid trematode in all the local animals examined, together with the artificial infection of fish and the large number of negative controls, is quite conclusive evidence that all the stages found belong to the same species of trematode.

Egg-miracidium Stages

The egg is more or less oval in contour but varies quite considerably in different specimens (Fig. 1), In a few it is distinctly constricted at the opercular end and the operculum is quite obvious; in others, it is an almost perfect oval and the operculum is seen with difficulty. Frequently there is a small thickening at the end opposite the operculum. In size it varies from 26μ to 32μ long and 18μ to 22μ wide, the mean size of a considerable number being 29μ by 20μ. In color it is light brown, and the outer surface has many honey-combed, irregular lines on it. When deposited in the intestine, it is not embryonated and I am unable to state the exact time necessary for the development of the miracidium. In the laboratory, in shallow water in Petri dishes, at least two weeks were required but further data on this are necessary.



Apophallus venustus

FIG. 1. *Outline drawings of four eggs showing variation in outline.*

The egg does not appear to hatch naturally, and specimens kept in the laboratory for almost a year did not hatch. As the pressure necessary to force off the operculum also invariably destroyed the miracidium, I have been

unable to ascertain details of this larval stage. It does not however, completely fill the egg shell, which also contains several large globules of a fat-like substance (Plate I, Fig. 2). Its general appearance is very similar to that of *Cryptocotyle* and *Opisthorchis*. The miracidium appears to hatch only after ingestion by the snail. Several snails, from a stock known to be parasite-free, were exposed to infection by placing them for a few hours in a Petri dish, the bottom of which was covered with washed feces containing eggs. The snails were then removed and placed in a clean aquarium jar and ultimately mother-redia were found in them. The snails unfortunately died at this stage, probably as the result of superinfection.

The culture of eggs was examined immediately after the removal of the snails and no hatched eggs were seen. This removes the possibility of some special stimulus, associated with the snail, being responsible for hatching, and leaves actual ingestion as the only alternative.

Snail Host (Plate I, Figs. 3, 4, 5)

As mentioned in the introduction, the only species of local snail found naturally infected was *Goniobasis livescens*. They are relatively abundant in parts of the Ottawa and St. Lawrence Rivers, near Ste. Anne de Bellevue, in areas where the necessary environmental conditions are found.

The genus has its centre of distribution in the southern United States, and LaRocque* is of the opinion that only two species occur in eastern Canada, *viz.*, *G. haldemani* in Lakes Erie and Huron and *G. livescens* in the lakes and rivers of the drainage area of the Great Lakes and St. Lawrence—except those emptying into the St. Lawrence below the salt water line. All the specimens of *Goniobasis* used in the present work appear to belong to this species.

Goniobasis livescens has a thick, solid, more or less spiral, elongated shell, with a subrhomboidal aperture, closed by a subspiral horny operculum. There is a tendency among conchologists to divide this species into varieties which correspond with differing habitats. Its general habitat is a boulder bottom or on stones with sandy spots. Its presence in the latter area can usually be traced by the deep furrows which it makes as it moves along the sand. It is generally found in from one to three feet of water, in situations exposed to the river current, where it feeds on desmids and diatoms.

Baker (1) describes its behavior in an aquarium as follows:

"It will glide up the side of the glass jar with as much celerity as a *Lymnaea* or *Physa*, though not as rapidly. When at rest on the bottom, it moves more or less slowly, pushing its foot forward a short distance and drawing the shell after it, a sort of stepping process. While in motion, the rostrum is constantly in motion searching for food, the action of the radula being plainly

* *Museum Assistant, Division of Paleontology, Geological Survey, Department of Mines and Resources, Ottawa.*

seen as it scrapes the stones and other objects. The mouth may be opened and the radula seems to be used in a lapping manner. The long tentacles are constantly in motion, being used as tactile organs feeling and testing all objects within reach. Sight is apparently deficient, the tentacles performing the function of this sense."

Our experiences with this species are fully in accord with Baker's.

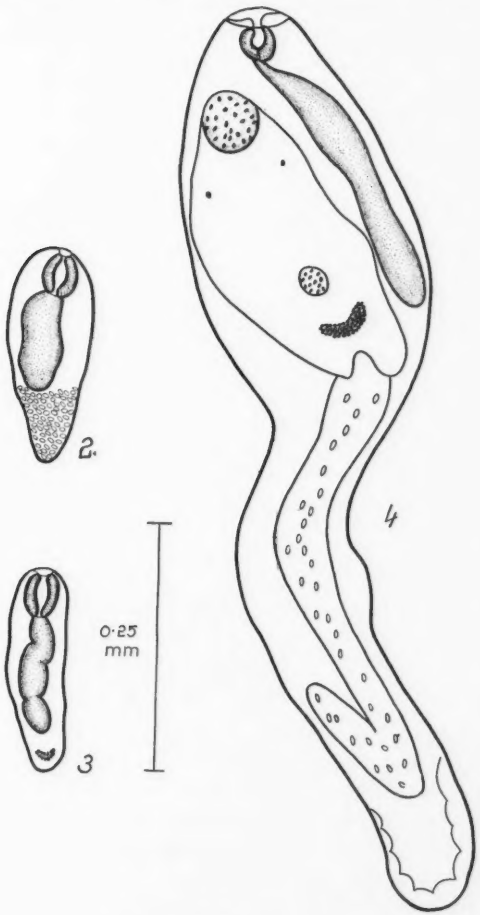
While *Goniobasis* occurs in a variety of places in the local waters, the most plentiful sources of supplies were found to be the Isles de la Paix on the St. Lawrence and the east end of Lynch Island, about two miles down the Ottawa River from Ste. Anne de Bellevue. Large numbers were collected from both these areas. All snails were examined for infection shortly after being brought into the laboratory and at intervals during the succeeding two months. The specimens that died were examined by crushing and searching under the binocular dissecting microscope. Living snails were placed individually in quarter-pint glass cream bottles, filled with water and examined with a hand lens for escaped cercariae 24, 48 and 72 hours later. Negative examples were placed in a stock jar, positive examples used as a supply of larvae. Negative examples were re-examined periodically and further segregated. In no case was any naturally infected snail found in the St. Lawrence islands. However, a large number of infected snails was found near Lynch Island in the Ottawa River; in one batch of 82 examples (part of a single day's collection) 26 infected snails were found—an infection rate of 31.6%. As such natural bird hosts as might exist or have been discovered are common to both areas, I can give no reason for this heavy infection rate or for the complete absence of any infection on a group of islands in the St. Lawrence, only four miles distant. In the spring of 1936, a large number of snails of this species was obtained from the Rideau River in Ottawa city. Two hundred of these were crushed and examined; two contained the cercariae of this trematode and two contained rediae and daughter rediae which may also belong to the same species, but which were too immature to identify.

Intra-molluscan Development

There has been little opportunity to examine the intra-molluscan development of the parasite and, as mentioned above, experimental infection was carried only as far as the mother-redia stage. The sporocyst stage has not been identified and all subsequent stages occur in the liver (Plate I, Fig. 6).

Young mother rediae were seen only in experimental infections, infected on October 30, 1935, and examined on November 16. At that stage of development, they are carrot-shaped organisms, about 0.22 mm. long (Fig. 2), with an almost spherical oral sucker at the blunt end, and a very large single caecum extending posteriorly for about two-thirds of the body length. The remainder of the body is filled with spherical cells. These rediae become much larger and are ultimately filled with daughter rediae (Fig. 3), which,

in their young stages, are small oval bodies with an oral sucker and a single caecum. The daughter rediae can move about by vermiform contractions of the body, but possess no hooks or spines. When fully mature (Fig. 4), they are elongated sausage-shaped bodies, with no appendages and apparently no functional birth pores. They contain a varying number of cercariae—from one to eight—rarely more (Plate I, Figs. 7, 8). These cercariae are fully developed morphologically before they leave the daughter rediae.



Apophallus venustus

FIGS. 2, 3 AND 4. FIG. 2. *Young mother redia.* FIG. 3. *Young daughter redia.* FIG. 4. *Mature daughter redia, containing a single cercaria. All drawn to same scale.*

Cercaria

The cercaria (Figs. 5, 6) is a very muscular larva and subject to great changes in body shape. This description is based on relaxed, living specimens, supplemented by sections and whole mounts of cercariae killed in formalin and stained with alum carmine. It has a long tail about one and one-half times the body length—sometimes a little longer, but never more than twice the body length. It is provided with a complete dorsal and an incomplete ventral fin of a very delicate structure (Plate II, Fig. 1). The dorsal fin originates as a very small fold close to the body and, gradually increasing in width, reaches its maximum about half-way down the tail; thereafter it becomes slowly reduced in size. It is continued around the ventral side to expand again to form a ventral fin, which however terminates about the mid-point of the tail. In a minority of specimens, there is a second ventral fin close to the body (shown as a dotted line in Fig. 5). The membranous fin is longer at its free edge than on its attached edge and it is, in consequence, undulated. This tail is very muscular and is attached, by a "ball and socket" mechanism to the posterior end of the body. It is very easily detached by its own rapid movement.

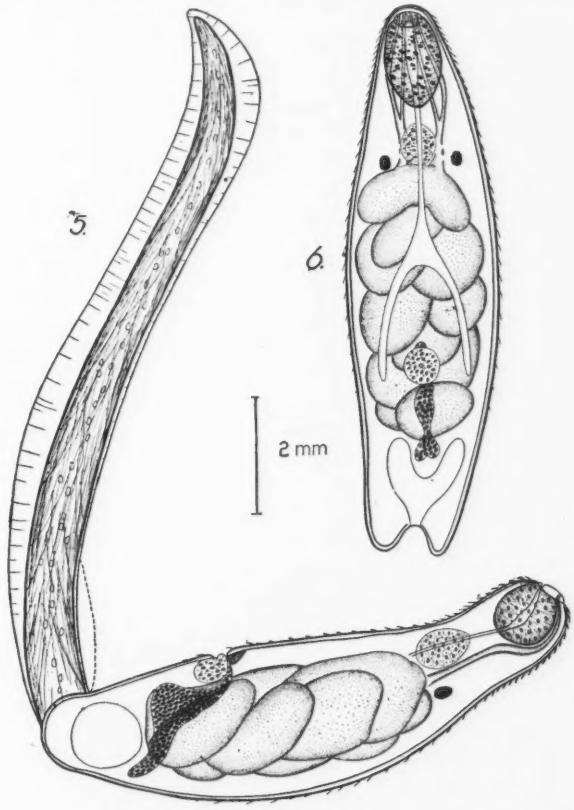
The body is about 0.10 to 0.13 mm. long and about one-third of this in width (Fig. 6). The oral sucker is spherical to oval in shape and only feebly muscular. It is about 20μ in diameter. It is sub-terminal in position and the mouth opening is towards the ventral surface. There are no hairs on the body. However, the whole surface of its anterior part to about its mid-point, is thickly covered with minute spines (as in the adult) except for a small area surrounding the termination of the oral sucker and just dorsal to it. The spines at the edge of this area are slightly larger than the rest. The spines on the posterior half of the body are few in number. The oral sucker is pierced by the oesophagus, which continues as a minute simple tube, to a point just in front of the middle of the body. There the tube bifurcates, and in one specimen the bifurcations were traced as far as the level of the ventral sucker. About mid-way between the bifurcation and the anterior end, the oesophagus is surrounded by an oval muscular pharynx.

Dorsal to the pharynx are two darkly pigmented eyespots of varying size.

The ventral sucker is rudimentary and appears to have no muscular fibres at this stage. It is however, provided with a small central depression. Just anterior to this is another small depression, surrounded by a mass of small cells—the rudiments of the genital sinus complex (Plate II, Fig. 4).

Passing from this point, dorsally and posteriorly, is a mass of cells, which stains deeply in preserved specimens and obviously represents the genital primordia. This mass (Plate II, Fig. 2) expands at about its mid-point and then commences to form two terminal swellings. Viewed from the side (Fig. 5) it is roughly "L" shaped. On the lateral margins of the body posterior to this are pigmented areas, and between these two areas lies the "Y" shaped excretory vesicle. I was unable to trace the excretory system in detail,

although a number of flame cells was observed in different parts of the body. Practically the whole of the centre of the body is filled with 16 large glandular cells which are readily demonstrated by vital staining. The ducts of these cells pass anteriorly in two medial bundles, which divide into four bundles of



Apophallus venustus

FIGS. 5 AND 6. FIG. 5. *Cercaria, side view.* FIG. 6. *Body of cercaria, from ventral aspect.*

four ducts each, just before the oral sucker is reached. These pass forward, in a dorso-lateral direction, to open on the spineless area just dorsal to the oral sucker.

This spineless area can be protruded slightly and this enables these glands to come into close contact with the skin of the fish and assist in penetration. Although there is no proof of the fact, it is probable that these glands secrete a histolytic ferment used in penetration. It is possible that a negative pressure from the oral sucker helps to maintain the larva in place during the time this

digestive process takes place; the ventral sucker appears to play no part in the action, at least in such specimens as were observed in the act under the microscope.

This cercaria, while possessing the same general characters as other members of this family, differs from them in detail. From the cercaria of *Cryptocotyle lingua* (which is the nearest relation of this species, the life cycle of which is known, and which develops in the marine gastropod, *Littorina littorea*), it differs in its shorter tail, smaller oral sucker, 16 instead of 18 glands, and in the presence of a rudimentary ventral sucker. (5)

The conditions of emergence of the cercariae are not yet fully understood. Temperatures within the limits prevailing naturally (18° C. to 24° C.) appear to have no effect. There was, however, a definite time influence which may be connected with light intensity. From a number of snails examined at hourly intervals during the day (10.00 a.m. to 12.00 midnight), it was found that none emerged before 4.00 p.m., a few between 5.00 and 8.00 p.m. and most between 8.00 and 10.00 p.m. Few emerged during the period between midnight and 10.00 a.m., and the optimum time appeared to be 8.00 to 9.00 p.m. These experiments were conducted at the beginning of October. All cercariae died within 48 hours of emergence in aquaria in the laboratory.

In jars in the aquarium, there is a definite tendency for the cercariae to congregate on the side next to the windows and towards the top of the water. They are most strongly attracted by direct light (on a southern window sill) and when the sun is obscured by a cloud they spread out and become less concentrated and less active. Artificial shadows have a similar effect. They are not only positively heliotropic, but are stimulated by light. This causes a reduction in their free life, and cercariae exposed in a north window do not live more than 24 hours, while those kept in complete darkness may survive for 48 hours.

They swim head foremost, with rapid figure-of-eight lateral lashings of the tail; this is done so rapidly that the entire larva becomes a blur. At the end of this rapid movement, the cercariae become suddenly quiescent and lie in the water with the body horizontal, the dorsal surface being downwards and the tail upwards at right angles to the body. In this position, they slowly sink in the water, suddenly to start rapidly swimming again. The swimming is generally vertically upwards and is generally carried out in short spurts of a few seconds each, the cercariae resting and falling a short distance between spurts. Only occasionally do they move in a diagonal direction.

In addition to moving through fluid by the action of their tails, the cercarial body is very muscular and it is able to crawl actively—especially after losing its tail—by worm-like contractions and expansions of the body.

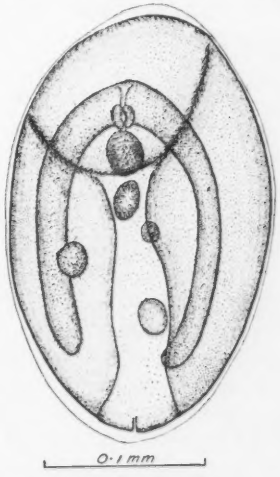
Contrary to Stunkard's experience with *Cryptocotyle lingua*, penetration of the fins by the cercaria can be observed with ease. Young catfish, about two inches long, are easily confined in a Petri dish of water which is placed on the stage of the binocular dissecting microscope. The cercariae, collected after emergence from the snail, swim about rapidly in the water for a short time. As soon, however, as one comes into contact with a fin—the body of the fish

appears to have little attraction for them—it holds itself against the skin by violent movements of its tail. These movements are so violent, that the tail is quickly detached. Meanwhile, the cercaria has been evidently digesting its way into the skin by means of the contents of its glands. It generally selects a position near the free edge of the fin, where the skin is thinnest, and within ten minutes it has entered. It generally succeeds in entering a blood vessel up which it moves by alternate contraction and expansion of its body, and it may be assumed that its spines are of considerable assistance in the process. Within 15 minutes of exposure to infection, the tail-less cercaria is seen disappearing into the body of the catfish and small drops of blood may be seen escaping from the open wound at the tip. This process has been repeatedly observed in these small fish. The young catfish are unable to stand the shock of too many penetrating cercariae and a number died from their effects. The bony dorsal and anal fins are the sites of election, although, less frequently, the tail fin also is attacked. Cercariae have never been seen attacking the fleshy dorsal fin of these fish, nor have metacercariae been found in the actual skin of any species of fish; they are always under it or in the muscles. The cercaria encysts in the musculature to become the metacercaria and it is further surrounded by an adventitious cyst formed by the fish itself.

The Metacercaria

The metacercaria (Plate II, Figs. 5, 6), after encystment in the fish, requires considerable time to become mature and infective. Experimental evidence is still lacking as to the exact time necessary, but it appears to be about four weeks. Once mature, probably it can live for a very long period—certainly over winter.

The mature metacercaria is virtually a young adult enclosed in a thin-walled, strong, elastic, hyaline cyst, which in turn is surrounded by a thick-walled fibrous cyst. This cyst is generally oval but varies considerably in size; the largest seen was 0.25 mm. long by 0.175 mm. wide. In this, folded on its ventral face (Fig. 7), lies the metacercaria, and in it the digestive system, ventral sucker, and genital rudiments can be observed. In addition, there is a mass of dark pigment in the excretory vesicle. In the young cyst, remnants of the penetration gland cells and ducts are still visible; these however gradually disappear. When freed from this cyst artificially, a young fluke (Plate III, Figs. 2, 3), about a half-millimetre long, is observed, in which the complete digestive tract, both testes, ovary and a large excretory vesicle filled with this black pigment can be seen.



Apophallus venustus

FIG. 7. *Metacercaria, still enclosed in cyst, but with fibrous outer cyst removed by digestion.*

Fish Hosts

No attempt was made to examine these fish for the presence of metacercariae (other than experimentally infected catfish and the catfish described later) and reliance was placed on experimental feeding of cats. These cats were all raised locally and had not previously been fed on fish. They were placed in cages and each one fed on a single species of raw fish, collected from the Ottawa River.

During 1935 and 1936 the following fish (4) were fed to individual cats:

Scientific name	Popular name	1935 experiments	1936 experiments
Lepisosteidae			
Lepisosteus osseus	Garpike	Positive	—
Catostomidae			
Catostomus commersonii	Sucker, common	Positive	Negative
Moxostoma aureolum	Sucker, red-horse	—	Negative
Cyprinidae			
Lvxilus cornutus	Dace	Positive	—
Cyprinus carpio	Carp	Positive	—
Ameiuridae			
Ameiurus nebulosus	Bullhead or catfish	Positive	Positive
Esocidae			
Esox lucius	Northern pike	Positive	Positive
Percidae			
Stizostedion vitreum	Wall-eyed pike (or Doré)	Negative	Positive
Perca flavescens	Perch	Positive	Negative
Centrarchidae			
Micropterus dolomieu	Small-mouth black bass	Positive	Positive
Eupomotis gibbosus	Common sunfish	Positive	Positive
Amblophites rupestris	Rock bass	Negative	Negative

Only seven red-horse suckers were available for feeding purposes in 1936, and so no conclusions can be drawn from the negative results. However, 323 perch were fed with no result in 1936 and the infection from these fish in the previous season was a light one. Forty-nine wall-eyed pike in 1936 gave also a very light infection and it seems reasonable to conclude that these are both "poor" hosts. The number of rock bass fed in 1935 was not recorded but it was large, while in 1936, 43 were fed; in no case did an infection result. This result is peculiar as the closely related small-mouth black bass is an excellent host and the sunfish a good one. The best host however was the common bullhead or catfish, and one cat fed on thirty of these fish yielded 5,882 flukes on post-mortem examination.

Ciurea(3), who has published a very detailed account of the metacercaria of the European *Apophallus donicus* as it occurs naturally in fish in Rumania, finds that it occurs in *Perca fluviatilis*, *Lucioperca lucioperca* and *L. volgensis*

as well as in *L. sandra, Percarina demidoffi, Acerina cernua, A. schraetster, Aspro streber* (among the Percidae) and in *Scardinius erythrophtalmus, Abramis brama* and *Blicca björkna* (among the Cyprinidae). In light infections, the metacercariae are found exclusively in the fins, while in heavy infections they are also found in the skin or under the scales. In the fins, they are generally in the interior rays and rarely in the membranes; the caudal and pectoral fins are mainly infected. In the skin, they are generally in the deep layers and only in massive infections are they superficial.

The distribution of the cysts of *Apophallus venustus* has only been studied in the catfish. A number of these fish was cut up into carefully separated and identified segments. Each portion was then separately subjected to artificial digestion in pepsin-hydrochloric acid-saline solution for 24 hours. This procedure did not digest away the cyst membrane and the cysts in each part of the fish were easily counted. Their distribution is shown in Fig. 8;

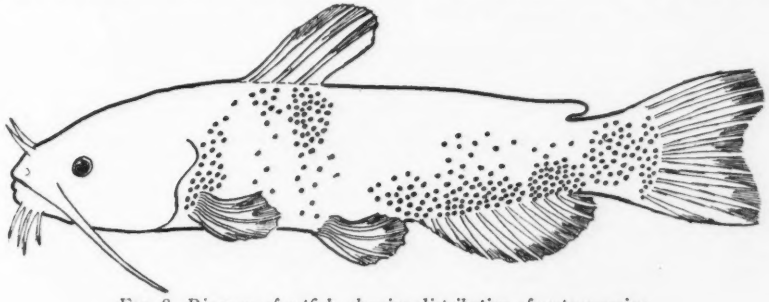


FIG. 8. *Diagram of catfish, showing distribution of metacercariae.*

each dot represents a cyst. The diagram is a composite one, representing the combined results of four separate fish. Most cysts are in the neighborhood of the unpaired, membranous fins (caudal, dorsal and ventral) but some are in the neighborhood of the paired fins as well. No cysts were found in the head region or in the dorsal region near the fleshy dorsal fin. All were in the actual musculature. This distribution agrees with the observations of the actual penetration of young catfish by the cercariae, which were only seen to enter by the membranous fins, never by other parts of the cuticle.

Definitive Hosts

In Canada, this parasite has been found in cats, dogs and raccoons *(Procyon lotor)* as well as in the great blue heron *(Ardea herodias herodias)*. No specimens were found in the following fish-eating birds taken locally under permit from the Department of the Interior:—

8 bitterns *(Botaurus lentigenosus)*.
2 kingfishers *(Megaceryle alcyon)*.
2 gulls *(Larus delawariensis* and *L. argentatus smithsonianus)*.
3 mergansers *(Mergus merganser americanus)*.
1 hooded merganser *(Lophodytes cucullatus)*.

The development in the final host has not been systematically studied. Although artificial digestion experiments suggest that the fluke excysts only after *intestinal* digestion, Ciurea found, on autopsy, that the metacercaria of *A. donicus* after excystment became ovigerous in two and a half days. In one critical experiment carried out here however, *eggs* were not passed by the cat until the twenty-fourth day after infection, although careful fecal examinations were made daily. However, from a cat fed on experimentally infected catfish, flukes containing eggs were recovered on autopsy, a week after infection.

Several infected cats retained in the laboratory over winter had lost their infection by spring, and it seems probable that the mature life of the trematode is limited to a few months in these animals, and that the infection is maintained in the metacercarial stage in fish. This hypothesis is strengthened by the fact that no infected snails were found locally before September, although large numbers were collected and examined throughout the summer.

Careful examination of the intestines of cats passing ova, showed that the majority of the trematodes were in the ileum, a few were in the jejunum and none in the duodenum. The villi are rather far apart in the latter part of the small intestine of the cat and this may partly account for the relatively short life of the parasite.

Malformations (Plate III, Figs. 4, 5, 6)

In several cases, malformed specimens were encountered, the principal alteration being in the number of the testes. In one case both testes were completely absent, while in several one or other testis was absent. It is interesting to recall that several genera in this family have normally only a single testis, while in others the position of the testes relative to each other is a variable character. In none of the present series was the latter character observed.

Acknowledgments

During the course of this work I have had the assistance not only of my colleagues but of numerous persons outside the Institute, and I wish to acknowledge particularly the help of Mr. J. B. Harkin and Mr. Hoyes Lloyd of the National Parks of Canada, Department of the Interior, who gave us authority to take several migratory birds; Mr. L. A. Richard, Deputy Minister, and Mr. B. W. Taylor, Department of Public Works, Game and Fisheries, Quebec, who gave us authority to net fish; Mr. A. LaRocque, National Museum of Canada, who identified the snails and Professor V. C. Wynne Edwards, McGill University, who identified the birds. I wish also to thank Professor Ciurea of Bucharest, Rumania, who gave me a number of specimens of *Rossicotrema donicum*, Dr. Price of the Bureau of Animal Industry, United States Department of Agriculture, who gave me the benefit of his experience in the systematics of this group, and Mr. J. J. Harpell, of Gardenvale, Que., who gave us permission to collect catfish and minnows from his pond.

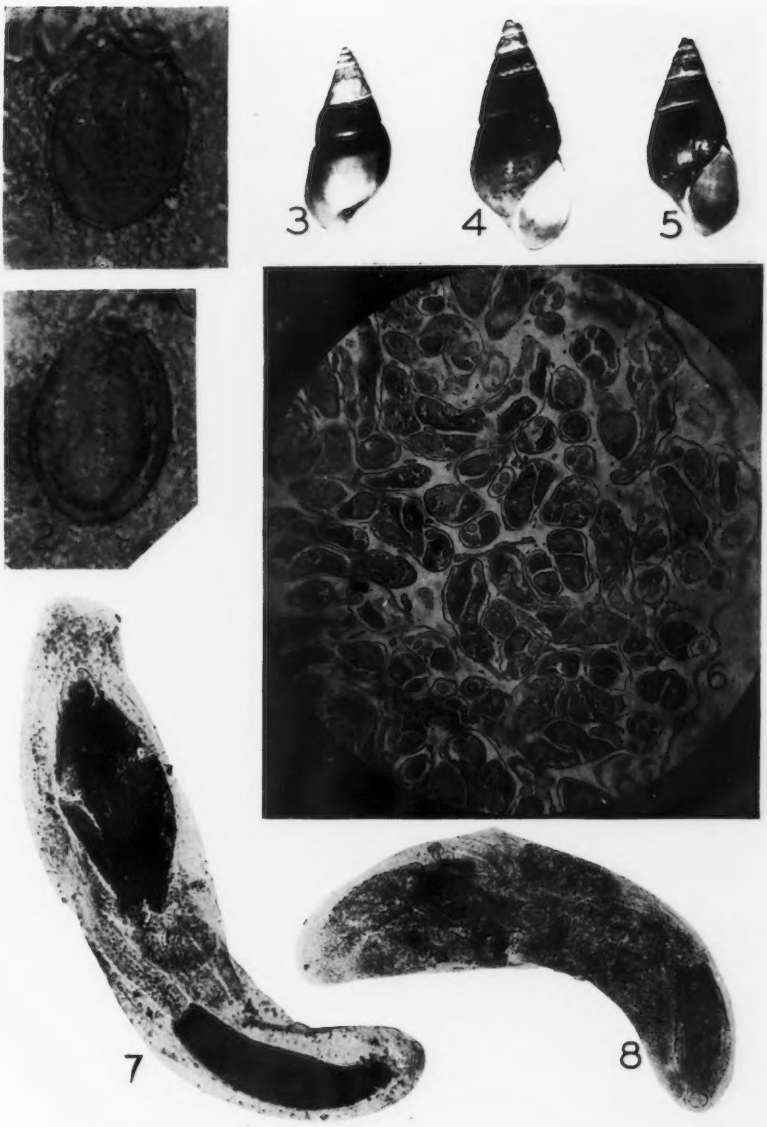
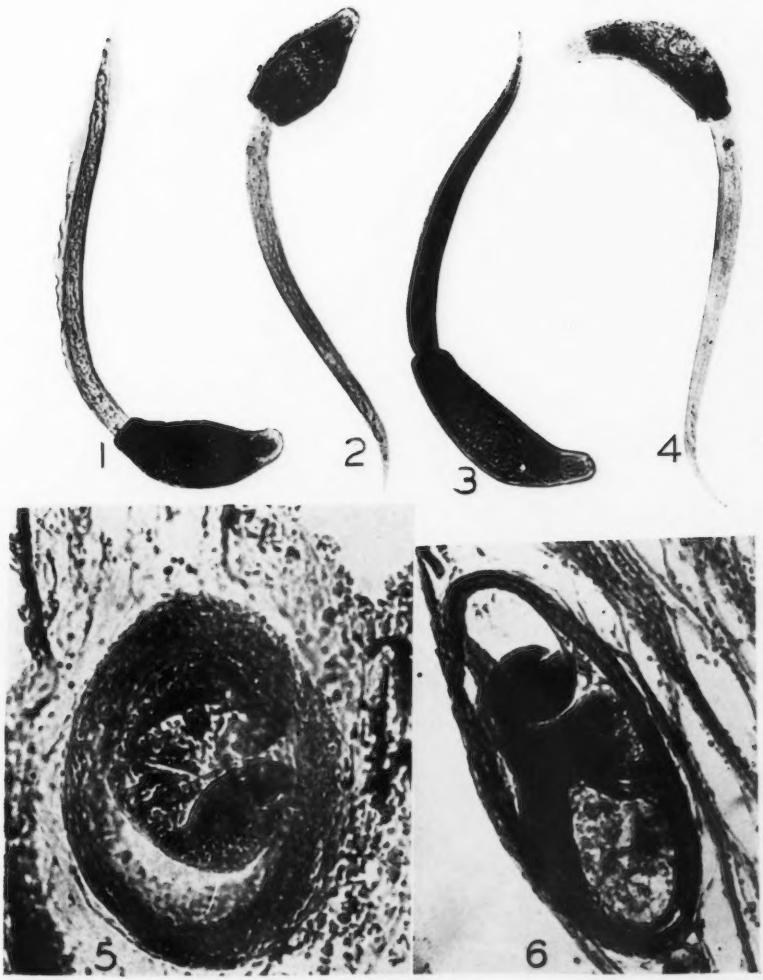


FIG. 1. *Egg of Apophallus venustus as passed in feces of cat (the operculum having been removed by pressure).* FIG. 2. *Egg containing miracidium.* FIGS. 3–5. *Goniobasis livescens.* FIG. 6. *Section of liver of Goniobasis livescens, showing daughter rediae and developing cercariae.* FIG. 7. *Daughter redia with two fully developed cercariae.* FIG. 8. *Daughter redia with eight cercariae.*



FIGS. 1–4. *Cercariae of Apophallus venustus.* FIG. 1. *Cercaria showing flanged tail, in position of rest in water.* FIG. 2. *Cercaria from ventral aspect showing eyespots, glands and genital primordia.* FIG. 3. *Cercaria from lateral aspect.* FIG. 4. *Cercaria from lateral aspect showing ventral sucker.* FIGS. 5–6. *Sections of metacercariae in muscles of catfish (Ameiurus nebulosus).*

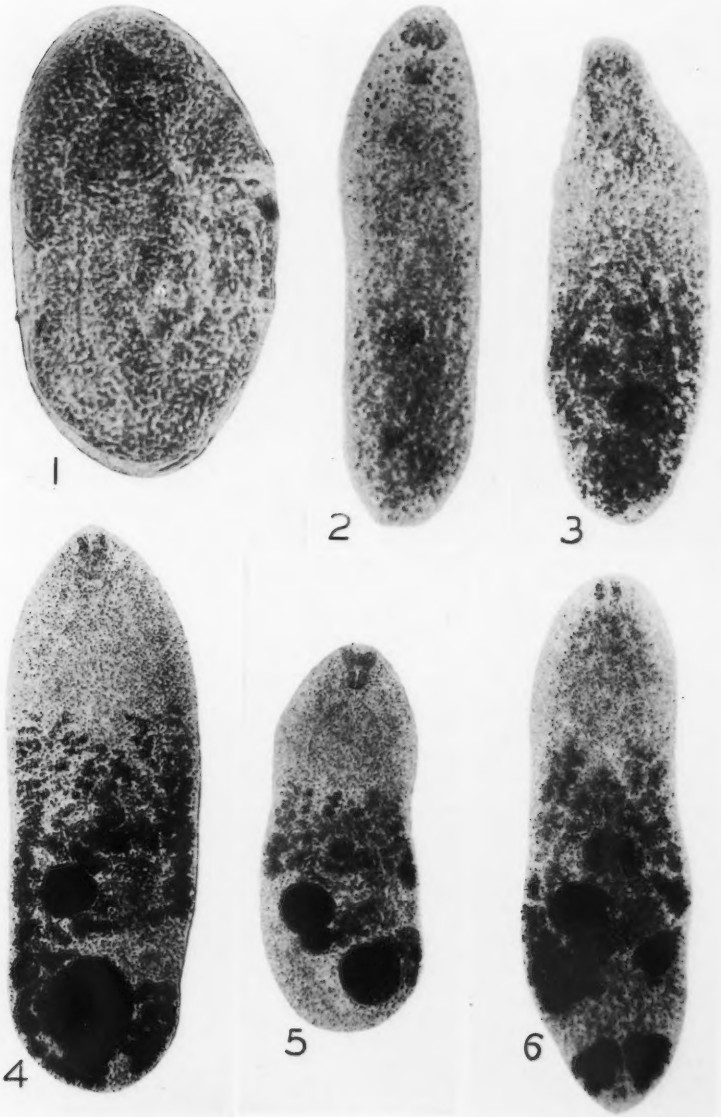
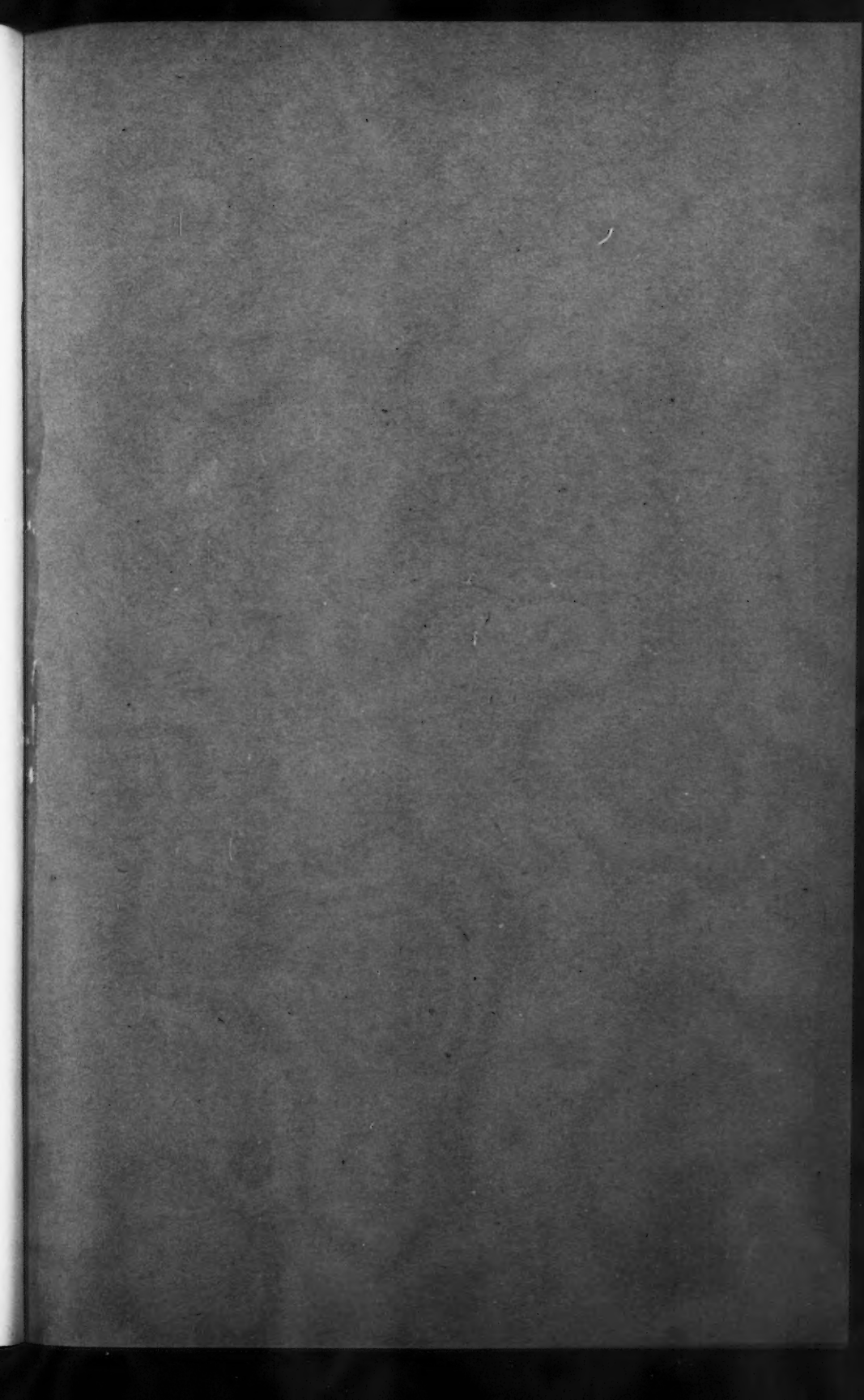


FIG. 1. *Metacercaria of Apophallus venustus with fibrous cyst removed.* FIGS. 2–3. *Immature adults from intestine of cat.* FIGS. 4–6. *Malformations in adults.* FIGS. 4 *and* 5 *show absence of a testis and* FIG. 6. *absence of both testes.*

References

1. BAKER, F. C. Fresh-water mollusca of Wisconsin. Part I. Gastropoda. Wisconsin Acad. Sci. pp. i-vi, 1-495. 1928.
2. CAMERON, T. W. M. Studies on the Heterophyid Trematode, *Apophallus venustus* (Ransom, 1920) in Canada. Part I. Morphology and taxonomy. Can. J. Research, D, 14 : 59-69. 1936.
3. CIUREA, I. Les vers parasites de l'homme, des mamifères et des oiseaux provenant des poissons du Danube et de la mer Noire. Arch. roumaines path. exptl. Microbiol. 6 : 4-134. 1933.
4. JORDAN, D. S., EVERMANN, B. E. and CLARK, H. W. Check list of the fishes and fishlike vertebrates of north and middle America north of the northern boundary of Venezuela and Colombia. Rept. U.S. Comm. Fisheries, 1928. Part II. 1-670. 1930.
5. STUNKARD, H. W. The life cycle of *Cryptocotyle lingua* (Creplin) with notes on the physiology of the metacercariae. J. Morphol. Physiol. 50 : 143-189. 1930.



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